Comparing Hybrid Nano-microfibrous Constructs of Plastic Compressed Collagen - Electrospun PLGA: Collagen Content Percentage as Variable

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TISSUE ENGINEERING

REGENERATIVE MEDICINE

ABSTRACT BOOK

Session No.: PL1 Plenary Speaker

From Basic to Clinical: Cell Sheet Tissue Engineering

Teruo Okano[1]

[1]Tokyo Women's Medical University

In 21st century, Tissue Engineering Regenerative Medicine is highly expected as innovative therapies, because it may regenerate the mass production volumes of cells and engineered tissues for advanced therapies. Thanks to tremendous research efforts by fusing biomedical scientists and physicians, we are getting close to enjoy effective regenerative medicine. However, we also face a serious conflict between its commercialization, and existing pharmaceutical regulations and a zero-risk approach, because we human kind have not established proper intelligence nor developed appropriate regulations yet for innovative regenerative medicine, which has to handle a large volume of living cultured cells or engineered tissues. Innovative consensus and intelligence have to be developed now for regulating tissue engineering regenerative medicine by advanced science-based evidence.

We have proposed this novel system of cells and cell-layers arrangement called "cell sheet engineering". Based on this break-through technology, we have initiated new project to promote "cell sheet engineering" to treat patients. We have initiated human clinical studies of cell sheet engineering therapy using oral mucosal cell sheet for treatment of cornea epithelium deficient disease, prevention of stricture and recovery from endoscopic submucosal dissection surgery for esophageal epithelial cancer and chondrocyte sheet for treatment for osteoarthritis of cartilage. Also we have succeeded in treating cardio-myopathy using myoblast cell sheet. From point of view cell sheet engineering with the intelligent surface is a highly promising tool for tissue engineering and regenerative medicine.

I am expecting active and professional discussion among the participants in TERMIS-AP from various aspects to create a new regulatory approach with our wisdom to optimize the balance between safety and efficiency for allowing regenerative medicine to move from bedside to industry in the world.

Session No.: PL2 Plenary Speaker

Implication and Application of Direct Cellular Reprogramming

Kam Leong[1]

[1]University of Columbia

Direct cell reprogramming, where differentiated cells are reprogrammed into another lineage without going through an intermediate stem cell-like stage, produces cells promising for regenerative medicine. It obviates the use of embryos and minimizes the risk of teratoma formation associated with the use of induced pluripotent stem cells. Direct reprogramming can also produce cells for disease modeling and drug screening. I will discuss our recent effort to convert human endothelial progenitors (hEPC) into induced smooth muscle cells (iSMC), hEPC into induced skeletal myocytes (iSkM), and human fibroblasts into induced cardiomyocyte-like cells (iSML). I will describe various approaches of achieving direct cell reprogramming using transcription factor overexpression, microRNA delivery, molecular pathway manipulation, and CRISPR/dCas9-based transactivation either separately or in combination.

Session No.: PL3 Plenary Speaker

Cartilage Engineering - from Bench to Bed-side

Yiling Cao[1]

[1]Shanghai Chiao Tung University

Functional repair of cartilaginous defects has long been a great challenge in reconstructive surgery. Cartilage engineering provides a promising alternative. However, despite over two decades of research endeavors, very few products have moved from bench to bed-side due to multiple bottlenecks in cell source, shape control, immune reaction, and long-term stability of the regenerated cartilage. Our group has long dedicated to addressing these issues through the following strategies: 1) Development of isolation, expansion, and chondrogenesis techniques for various chondrogenic cells; 2) Establishment of in vitro regeneration system to reduce immune reaction and ensure stable cartilage regeneration; and 3) Combination of laser scanning, computer aided design and manufacturing system, and 3D printing techniques for shape control; We also established multiple pre-clinical big animal models to investigate the efficacy of these strategies. Based on these, we made several breakthroughs for clinical translation of tissue engineered cartilage such as trachea and auricle, and recently we successfully realized the first-in-human trial of external ear reconstruction based on tissue engineered human-ear-shaped cartilage. These achievements would promote clinical application of engineered cartilage as well as other types of tissue.

Session No.: PL4 Plenary Speaker

How's the Hematopoietic Stem Cell Regulated by Bone Marrow Niche?

Hyo-Soo Kim[1]

[1]Seoul National University Hospital

Hematopoietic stem cell (HSC) transplantation is the most widely used regenerative therapy for a variety of life-threatening hematologic diseases. An essential factor in successful transplantation includes fine adjustments in hematopoiesis as determined by long term repopulating hematopoietic stem cells (LT-HSCs) residing in a specialized microenvironment in the BM. In addition to signature molecules in LT-HSCs, other important factors that may influence HSC quiescence versus proliferation include various environmental factors in the BM, such as angiopoietin-1, osteopontin, stromal derived factor-1, thrombopoietin and hypoxia inducible factor-1 alpha (HIF-1). We recently reported that K factor is a hypoxia-responsive gene and that its expression is induced in ischemic tissues. Thus, we investigated the role of K factor in regulating HSCs in the BM niche. Interestingly, K factor is predominantly expressed in LT-HSCs, but is expressed only at a trace amount in short-term repopulating-HSCs (ST-HSCs) or multipotent progenitor cells (MPPs) located in the BM niches. To examine whether K factor affects the biological behaviors of LT-HSCs, we generated the K factor-knockout (KAI1-/-) mice. Although the number of ST-HSCs and MPPs was normal, the number of LT-HSCs was selectively decreased because LT-HSCs lost quiescence and entered the cell cycle.

(Jin Hur Hyo-Soo Kim. CD82/KAI1 maintains the dormancy of long-term hematopoietic stem cells through interaction with DARC-expressing macrophages. Cell Stem Cell 2016, March)

Session No.: PL5 Plenary Speaker

The Bionic Man: Not too Far Away!

Paul Cederna[1]

[1]University of Michigan

It has been 35 years since Luke Skywalker (Star Wars) received a prosthetic hand controlled by his peripheral nerves. Unfortunately, this peripheral nerve interface has not been achieved in reality largely due to the difficulty recording multiple independent efferent motor control signals from a nerve inside a moving arm. The current best option is targeted muscle reinnervation (TMR), which moves divided nerves into alternate muscles that then function as signal amplifiers. This has worked very well and has provided a significant advance to our current approaches for prosthetic control. Our group has taken this strategy one step further by performing regenerative peripheral nerve interfaces (RPNI), which consist of a skeletal muscle graft placed on the end of a surgically subdivided nerve (nerve fascicle) to provide more control signals for dexterous hand motion. In addition, the RPNI's can be used as a peripheral nerve interface strategy to provide high fidelity sensory feedback from the terminal device to the sensory afferents. This closed loop neural control strategy has facilitated recordings of efferent motor nerve action potential for motor control and afferent nerve stimulation for sensory feedback, over prolonged periods of time with highly favorable signal-to-noise ratios. To date, we have tested the safety and signal quality thoroughly in over 500 animals, two non-human primates, and one human. In this presentation, I will share our last 10 years of research developing this novel peripheral nerve interface and discuss the future potential of this disruptive technology.

Session No.: AL1 Excellence Achievement Award

Cell Behavior on Polymer Surfaces and Well-defined Scaffolds

Hai Bang Lee[1]

[1]Ajou University, Dept. of Molecular Science & Technology

"Cell Behavior on Polymer Surfaces and Well- defined Scaffolds" Hai Bang Lee, Ph.D Professor, Ajou University, Department of Molecular Science and Technology, Suwon, Korea Emeritus Scientist, Korea Research Institute of Chemical Technology, Daejeon, Korea ABSTRACT The importance of polymeric biomaterials has been recognized in biomedical research for over four decades. The response of biomaterials in a biological environment is characteristically associated with their surface properties. The modification of polymeric biomaterials by various surface treatments by various surface treatments has become an active topic in surface engineering. A number of research group have focus on the preparation of surfaces with a gradually varying chemical composition along one dimension. Such a “gradient surfaces” is of particular interest for basic and applied studies of the interactions between biological species and surfaces as the dependence of a selected property, such as wettability, on composition, can be examined in a single experiment on one surface. In this presentation, we, describe the preparation and characterization of gradient polymer surfaces, presenting overview of the progress on over two decades in my laboratory. The introduction and characterization of biofunctional groups on gradient surface surfaces will be presented. Finally, we concentrate on the interaction of these surfaces with biological species, such as proteins and cells, important in the understanding of the basic science for biomedical applications.1.2 An appropriate cellular response to implanted surfaces is essential for tissue regeneration and integration. We investigated how human bone marrow stromal cells (hBMSCs) and human adipose-derived stem cells (hADDSCs) respond to scaffold substrates.3, 4 References 1. M. S. Kim, Kang, G., Lee, H.B., Prog. Polym. Sci., 2008. 33, 1338-164. 2. M. S. Kim, et al., Biomaterial 2008, 28, 5137-5143 3. K. S. Kim, et al., Tissue Eng. 2009, Part A, 15(10) 3201-3209 4. H. H. Ahn, et al., Tissue Eng. 2009, Part A, 15(7) 1821-1832

Session No.: AL2 2016 Outstanding Scientist Award

Injectable Cell Delivery Constructs for Myocardial Tissue Engineering

Hsing-Wen Sung[1], Chieh-Cheng Huang[1], Ding-Yuan Chen[1], Chun-Wen Hsiao[1], Wen-Yu Lee[1], Yen Chang[2]

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Cell transplantation via direct intramuscular injection is a promising therapy for patients with ischemic diseases. However, following injections, retention of transplanted cells in engrafted areas remains problematic, and can be deleterious to cell-transplantation therapy. In this presentation, a thermo-responsive hydrogel system composed of aqueous methylcellulose (MC) blended with phosphate-buffered saline is constructed to grow cell sheet fragments and cell bodies for the treatment of ischemic diseases. The as-prepared MC hydrogel system undergoes a sol–gel reversible transition upon heating or cooling at approximately 32 ℃. Via this unique property, the grown cell sheet fragments (cell bodies) can be harvested without using proteolytic enzymes; consequently, their inherent extracellular matrices (ECMs) and integrative adhesive agents remain well preserved. In animal studies using rats and pigs with experimentally created myocardial infarction, the injected cell sheet fragments (cell bodies) become entrapped in the interstices of muscular tissues and adhere to engraftment sites, while a minimal number of cells exist in the group receiving dissociated cells. Moreover, transplantation of cell sheet fragments (cell bodies) significantly increases vascular density, thereby improving the function of an infarcted heart. These experimental results demonstrate that cell sheet fragments (cell bodies) function as a cell-delivery construct by providing a favorable ECM environment to retain transplanted cells locally and consequently, improving the efficacy of therapeutic cell transplantation.

Session No.: SL1-01 Special Speaker

Drug and Gene Delivery by Self-assembled Supramolecular Nanosystems

Kazunori Kataoka[1]

[1]University of Tokyo

Nanotechnology-based medicine (Nanomedicine) has received progressive interest for the treatment of intractable diseases, such as cancer, as well as for the tissue regeneration through the delivery of therapeutic transcriptional factors. This presentation focuses present status and future trend of self-assembled nanosystems from block copolymers for the delivery of therapeutic compounds feasible for tissue regeneration. Nanosystems with 10 to 100 nm in size can be prepared by programmed self-assembly of block copolymers in aqueous entity. Most typical example is polymeric micelles with distinctive core-shell architecture. Compared with conventional formulations, such as liposomes, polymeric micelles have several advantages, including controlled drug release, tissue penetrating ability and reduced toxicity. Critical features of the polymeric micelles as drug carriers, including particle size, stability, and loading capacity and release kinetics of drugs, can be modulated by the structures and physicochemical properties of the constituent block copolymers. The development of smart polymeric micelles that dynamically change their properties due to sensitivity to chemical or physical stimuli is the most promising trend toward nanomedicines, directing to the targeting therapy with high efficacy and ensured safety. Versatility in drug incorporation is another feasibility of polymeric micelles. Polymeric micelles loaded with mRNA have been successfully formulated with relevant properties for nanotherapeutics, such as penetrability into diseased sites in the body to reveal significant expression of therapeutic proteins without any severe immunological responses. Indeed, our recent studies revealed in vivo therapeutic efficacy of mRNA-loaded polymeric micelles for the treatment of smell-dysfunction and osteoarthritis through in situ expression of BDNF and transcription factor (RUNX-1), respectively. These results demonstrate the promising features of polymeric micelles as platform nanosystems for tissue regeneration through the selective delivery of a variety of mRNA encoding therapeutic proteins.

Session No.: SL1-02 Special Speaker

Drug Delivery Technology to Realize Tissue Regeneration

Yasuhiko Tabata[1]

[1]Kyoto University

A new therapeutic trial based on the natural potential of body to induce tissues and organs regeneration, has been recently expected. To realize this tissue regeneration therapy, cell therapy and tissue engineering have been investigated. The idea of tissue engineering is to artificially create a local environment which enables cells to enhance their proliferation and differentiation, resulting in cell-induced tissue regeneration. If a key growth factor is supplied to the right place at the right time period and concentration, the body system will initiate to physiologically function, resulting in the natural induction of cell-based tissue regeneration. One practically possible way to enhance the in vivo therapeutic efficacy of growth factor is to make use of drug delivery system (DDS) technology. We have designed biodegradable hydrogels for the controlled release of growth factors and experimentally and clinically succeeded in the regeneration and repairing of various tissues. This release system can be combined with cells or/and the cell scaffold to promote the therapeutic efficacy of tissue regeneration. In addition, a chemokine is released to enhance the in vivo recruitment of stem cells to a target site to be regenerated, followed by the release of growth factor to activate the cells recruited thereat for tissue regeneration. If a fibrotic tissue can be digested by any DDS method to loosen or disappear, it is highly expected that the tissue is regenerated and repaired based on the natural healing potential of the surrounding healthy tissue. In this paper, several concrete experimental data on promoted tissue regeneration are presented to emphasize scientific and clinical significance of drug delivery technology to realize tissue regeneration therapy.

Session No.: SL1-03 Special Speaker

Polymer Matrices with Functional Gradients in Tissue Engineering

Jin Ho Lee[1]

[1]Hannam University

The Many biological processes in the body are mediated by physical or biochemical signal gradients. There are many kinds of signal gradients in the body, including chemotaxis, heptotaxis, and mechanotaxis. These signal gradients induce the homing or differentiation of various stem cells to specific target cells and thus can regenerate various tissues or organs. So, if we can control these physical or biochemical signals and their gradients, we may be able to have more control cell behaviors and enhance tissue formation. We have tried to fabricate various 2D and 3D physical and biochemical gradients for differentiation of stem cells to regenerate target tissues. Among the polymer matrices with these signal gradients, pore size, stiffness, and growth factor gradients to control stem cell differentiations and target tissue regeneration will be discussed in this presentation.

Session No.: SL2-01 Special Speaker

The Construction and Application of Stem Cell-based Tissue Engineered Nerve

Xiaosong Gu[1]

[1]Nantong University

Peripheral nerve defect leads to a high disability rate. The functional reconstruction of injured nerve is an important issue in regenerative medicine. Based on the mechanisms underlying peripheral nerve regeneration and the properties of biomaterials, we have developed a natural chitosan nerve graft that biodegrades at a controlled speed, for repairing injured peripheral nerve. Chitosan conduit satisfies the biological and physicochemical requirements of physical scaffold of tissue engineered nerve graft, such as biocompatibility, biodegradability, permeability, biomechanical properties, surface properties, and low immunogenicity, and thus is used in our laboratory as an ideal neural scaffold. The application of chitosan conduit allows sufficient exchange of nutrient and gas, benefits the growth of blood vessel, and promotes and guides the oriented growth of nerve fibers. The biodegradation product of chitosan, chitooligosaccharide, promotes the adhesion and growth of neural cells as well as the proliferation and migration of Schwann cells. To advance bone marrow mononuclear cell-based cell therapy into the clinic for peripheral nerve repair, we developed a new design of tissue-engineered nerve grafts, which consist of a chitosan/fibroin-based nerve scaffold and bone marrow mononuclear cells serving as support cells. Based on dog experiments, the results were observed that the sciatic nerve truck had been reconstructed with restoration of nerve continuity, functional recovery for conducting electrical impulses and transporting materials, and re-innervations of target skeletal muscle, which improved the locomotion activities of the operated limb. With the approval from the Chinese State Food and Drug Administration (SFDA) into clinical trials, we have launched a prospective randomized controlled multicenter study for the clinical use of chitosan-based nerve grafts, getting satisfying functional recovery.

Session No.: SL2-02 Special Speaker

Tissue Engineering of Lung Organoids for Disease Modeling

John Yu[1]

[1]Chang Gung Memorial Hospital

We have deciphered the glycoprotein expression pattern in lung cells by glycoproteomic strategy and identified surface markers for prospective isolation of lung stem/progenitor cells (LSCs) which reside in the bronchoalveolar junction of lungs. These isolated LSCs have the ability not only for self-renewal and differentiation, but also for in vivo engraftment and repair of lung tissues (Nature Nanotech 8:682 2013). Recently, we found that Fut8 (α1, 6-fucosyltransferase) activity in the isolated LSCs was approximately 14 fold greater than that in the differentiated type II and I alveolar cells. Given the inherent limitations for mechanistic studies using animal model, we developed organotypic cultures for lung alveologenesis from isolated LSCs to elucidate molecular processes involved in lung disorders associated with decreased core-fucosylation. We have thus established a unique LSC-derived 3D organotypic tissue model to recapitulate lung alveologenesis for formation of 3D alveolar sacs in normal lung and the structural features of alterations reminiscent of changes in chronic obstructive pulmonary disease (COPD). Finally, an unexpected finding of the involvement of Fut8 in type 2 epithelial-mesenchymal transition (EMT) in culture by which epithelial LSCs are converted to fibroblastic phenotypes upon differentiation has also prompted us to investigate its new implications in small airway fibrosis.

Session No.: SL2-03 Special Speaker

Building Complex 3D Biomimetic Elastic Structures with Assembled Human Tropoelastin Components

Anthony Weiss[1]

[1]University of Sydney

We have defined synthetic biology approaches that utilize the dynamics of individual human elastic protein molecules to give unprecedented control over spatial assembly, with the goal of building macromolecular 3D structures for tissue assembly and repair. The mechanical stability, elasticity, inherent bioactivity, and self-assembly properties of elastin make it a highly attractive and powerful technology for the fabrication of versatile biomaterials. Our unique ability to make and engineer the key elastin component, tropoelastin, drives our precise control of physicochemical and organizational performance, and further broadens the diversity of elastin-based applications. Neovascularization in vitro and in vivo was conducted in mice and pigs. Computer-based predictive biomaterials modeling has accelerated our design of these 3D protein constructs, and gives us control over higher-order cross-linking, efficient assembly and directed cell interactions. Elastic fibers are integral to the extracellular matrix of vertebrate tissues such as blood vessels, skin and lungs. Tropoelastin materials accelerate angiogenesis in wound healing in small and large animal models. We are using our natural-based composites in diverse fabrication processes to manufacture 2D and 3D materials including fibers, gels, sheets and tubes. The extraordinary tunability of these elastin constructs means we can use them in a range of biomedical and tissue engineering applications that encompass cell encapsulation, vascular repair, nerve regeneration, wound healing and accelerated tissue repair. REFERENCES: Yeo et al.. (2016). Science adv. (2016) 2, e1501145. Aghaei-Ghareh-Bolagh et al. (2016) Curr. Opin. Biotech, 39, 56-60. Annabi et al. (2016) Adv. Mater. 28, 40-49. Wang et al. (2015). Adv. Health. Mater. 4, 577-584. ACKNOWLEDGEMENTS: National Institutes of Health EB04283. National Health and Medical Research Council. Australian Research Council. Wellcome Trust 103328.

Session No.: SL3-01 Special Speaker

Bioinspired and Biomimetic Tissue Engineering Approaches for the Regeneration of Different Tissues

Rui L. Reis[1]

[1]University of Minho

The selection of a proper material to be used as a scaffold or as a hydrogel to support, hold or encapsulate cells is both a critical and difficult choice that will determine the success of failure of any tissue engineering and regenerative medicine (TERM) strategy.
We believe that the use of natural origin polymers is the best option for many different approaches that allow for the regeneration of different tissues. In addition to the selection of appropriate material systems it is of outmost importance the development of processing methodologies that allow for the production of adequate scaffolds/matrices.
Furthermore an adequate cell source should be selected. In many cases efficient cell isolation, expansion and differentiation methodologies should be developed and optimized. We have been using different human cell sources namely: mesenchymal stem cells from bone marrow, mesenchymal stem cells from human adipose tissue, human cells from amniotic fluids and membranes and cells obtained from human umbilical cords.
The potential of each type of cells, to be used to develop novel useful regeneration therapies will be discussed. Their uses and their interactions with different natural origin degradable scaffolds and smart hydrogels will be described.
Several examples of TERM strategies to regenerate different types of tissues will be presented.

Session No.: SL3-02 Special Speaker

New Application for Chemeric Proteins Template Hybrid Materials in Regenerative Medicine

Shengmin Zhang[1]

[1]Huazhong University of Science and Technology

We designed a series of chimeric molecular templates made of two proteins, silk fibroin (SF) and collagen, silk fibroin and albumin (ALB), to synthesize HA nanoparticles with controlled shapes.The success in controlling the shape of HA nanoparticles allowed us to further study the effect of the shape of HA nanoparticles on the fate of rat mesenchymal stem cells (MSCs). We found that the nanoparticle shape had a crucial impact on the cellular uptake and HA nanospheres were internalized in MSCs at a faster rate. Both HA nanospheres and nanorods showed no significant in fluence on cell proliferation and migration. However, HA nanospheres significantly promoted the osteoblastic differentiation of MSCs in comparison to HA nanorods. Our work both in vitro an in vivo suggests that a chimeric combination of promoter and inhibitor proteins is a promising approach to tuning the shape of nanoparticles. It also sheds new light into the role of the shape of the HA nanoparticles in directing stem cell and tissue regeneration.

Session No.: SL3-03 Special Speaker

Silk Biomaterials for Regenerative Medicine

James Goh[1], Thomas Teh[1]

[1]National University of Singapore

The progress of different fields within biomedical engineering and life sciences has largely been biologically inspired. This is especially so in the field of tissue engineering and regenerative medicine, whereby researchers have looked at the works of nature for inspiration in strategies and design for scaffold materials and architectures of specific tissues. These nature-inspired materials are largely based on biopolymers and silk fibroin has shown to be an excellent example for regenerative medicine. Due to its unique molecular and supra-molecular structure, its customizable ligands-based bioactivity, its ability to self-assemble and its ability to be manipulated into various forms and structures, there has been much interest in its application as a biomaterial. There exist an array of techniques to process silk fibroin into various forms with customizable mechanical and biological properties, to provide the necessary cellular, architectural and chemical cues for the specific tissue types. The material can be processed into powders, films, gels, sponges, foams, yarns, knitted and woven mats for various interesting tissue engineering applications. Numerous researches, including our group, have looked into applying the material in regeneration of tissues such as bone, cartilage, tendon/ligament, intervertebral discs, skin and cardiovascular tissues. However, limitations persist in its widespread use due to source-based variations and lack in standardization of processing protocols, which leads to challenges in controlling the final consistency and quality of the product.

Session No.: S01-01 Keynote Speaker

Vascularization of Hydrogels in Tissue Engineering

Eric M. Brey[1]

[1]Illinois Institute of Technology

The circulatory system plays a number of vital roles in regenerating and functioning tissues. It supplies oxygen and nutrients, removes wastes and is a source of multiple cell types required to respond to changing physiological conditions. For nearly every tissue engineering and regenerative medicine (TERM) application, the ability to enhance, regenerate or engineer new tissues requires spatial and temporal control over the process of vascularization. While vascularization is being studied in a number of physiologic and pathologic processes, TERM applications present distinct challenges. For example, unique microenvironmental conditions result from biomaterial and cell combinations used in TERM applications that are not encountered in any other system. In addition, clinical applications require vascularization of large tissue volumes within time frames that are much lower than those found during vascularization in development and typical physiologic processes. These requirements place significant constraints on the design of TERM therapies. The study of vascularization in TERM applications is a complex and growing field. The goal of this presentation is to describe research focused on vascularization of hydrogels for TERM applications.

Session No.: S01-02 Keynote Speaker

Different Strategies of Adipose Tissue Engineering

Ming-Huei Cheng[1], Eric M. Brey[2], Hui-Yi Hsiao[3]

[1]Chang Gung Memorial Hospital
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[3]Center for Tissue Engineering, Chang Gung Memorial Hospital

Autologous fat grafting has been applied to the face cosmetic surgeries and also has been successfully used in reconstruction of head and neck, breast, trunk, and extremities. Many studies suggested that the survival rate of fat graft transfer is still unpredictable from 20 to 90 percent. In large volume of fat grafting without pedicle, the central portion of the injected fat graft tissue could suffer from the lack of nutrients supplied by blood leading to fat necrosis, calcification, liponecrotic cysts and abscess. Different strategies have been studies for years to promote adipogenesis and angiogenesis to promote engineered adipose tissue, acting as an alternative option for fat grafting . Numbers of synthetic and natural biomaterials have been investigated for adipose tissue engineering. Adipose-derived hydrogel containing basement membrane proteins were extracted from subcutaneous adipose tissue. Significant adipose formation were observed when the hydrogels were implanted in a rat epigastric pedicle bundle. The similar effect also found in dermis-derived hydrogel. Besides promoting in adipogenous, introducing vascular network into engineered adipose tissue is another alternative way to solve the fat survival issue. While pre-treating a fat transplant recipient site with negative pressure has shown promise for increasing the fat survival rate. The effect of negative pressure treatment on fat grafting recipient sites in a porcine model has found to increase the epidermis thickness which was due to cell proliferation in the epidermis. Furthermore, there was a more than two-fold increase in the vessel density and significant increase in many angiogenesis factors, such as FGF-1, VEGF and PDGF-bb. The results indicate the effect of negative pressure on fat survival is due to promotion on vascularization. The combination of these strategies could lead to generate applicable engineered adipose in clinical use.

Session No.: S01-03 Invited Speaker

Robust Biopolymer-based Supramolecular Hydrogels via the Host-guest Macromer (HGM) Approach for Preparing for Cartilage Repair

Liming Bian[1], Kongchang Wei[1], Qian Feng[1]

[1]The Chinese University of Hong Kong

Although biopolymer-based chemical hydrogels, with biopolymers covalently crosslinked, have been widely used as scaffolds for tissue engineering due to good stability, their permanent network structures and brittleness limit their applications in repairing load-bearing tissues, such as cartilage. In contrast, biopolymer-based supramolecular hydrogels, which are usually formed via self-assembly of physically interacting biopolymers, are usually weak as shown in "inverted vials" instead of freestanding 3D constructs and less stable than chemical hydrogels. Herein, we describe a novel host-guest macromer (HGM) approach for preparation of biopolymer-based freestanding supramolecular hydrogels. Host-guest macromers are formed by molecular self-assembly between adamantane functionalized hyaluronic acid (ADxHA) guest polymers and mono-acrylated beta-cyclodextrins (mono-Ac-CD) host-monomers. Supramolecular hydrogels are readily prepared by UV-induced polymerization of the pre-assembled host-guest macromers. Such hydrogels are solely crosslinked by in situ formed multivalent host-guest nano-clusters, and show significantly reinforced mechanical properties yet still retain desirable supramolecular features. They can self-heal and be re-molded into freestanding 3D constructs which afford effective protection on the encapsulated stem cells during the compression re-molding, making them promising carriers for therapeutic cells that can quickly adapt to and integrate with surrounding tissues of the targeted defects. We demonstrate that such hydrogels not only sustain extended release of encapsulated proteinaceous growth factors (TGF-beta1) but also support chondrogenesis of the human mesenchymal stem cells (hMSCs) and promote cartilage regeneration in a rat model. Lastly, we have developed a series of supramolecular hydrogels with unique properties such as resilient mechanical property, fast relaxation, self-healing, bioadhesiveness, injectability, and promoting recruitment of endogenous cells. These hydrogel properties are not only desirable for potential clinical applications of these hydrogels but also useful for studying the effect of microenvironmental mechanical cues on stem cell behaviors.

Session No.: S01-04

Biomimetic Collagen-targeting Mussel Protein Hydrogel for Scarless Skin Regeneration

Eun Young Jeon[1], Bong-Hyuk Choi[1], Hyung Joon Cha[1]

[1]Pohang Unsiversity of Science and Technologh

Skin scarring from surgical incisions or burn injuries is a major clinical challenging problem, with high psychological and aesthetic concern. Wound healing for adult skin involves the orchestration of complex interactions between numerous cells, extracellular matrix, and signaling molecules, leaving scar tissues with abnormal restoration and organization of tissue components, especially collagen. Because collagen reorganization in tissue remodeling is a critical determinant of dermal scarring for deep skin injures, development of collagen-targeting therapeutic can improve healing rate and quality, subsequently inhibiting scarring. Inspired by well-known influences of decorin, a collagen-targeting small leucine-rich proteoglycan (SLRP), in skin architecture and wound healing, we designed a new type of mussel-adhesive protein (MAP) by incorporating collagen-targeting peptide, using decorin as a model. This new collagen-targeting MAP functioned to specifically bind to collagen and delay collagen fibrillogenesis in vitro, similarly to decorin. Moreover, animal experiments using a rat excisional model demonstrated that the treatment of collagen-targeting MAP hydrogel resulted in prompt wound regeneration and improved collagen architecture with fibril diameter and distribution similar to those of healthy skin by visual, histological, and TEM analysis, Collectively, our evidence suggests that this collagen-targeting MAP hydrogel system would represent a promising therapeutic agent as a biomimetic approach aiming at improving wound regeneration and alleviating scarring in adult skin.

Session No.: S01-05

Design of a Sugar-responsive Gelatin Hydrogel as a Sacrificial Template to Create Vascularized Channel Structures in Collagen Gels

Masaya Yamamoto[1], Seiji Muratani[1], Kosuke Arimoto[1], Yasuhiko Tabata[1]

[1]Institute for Frontier Medical Sciences, Kyoto University

The objective of this study is to design a sugar-responsive gelatin hydrogel as the sacrificial template that can be removed by the sugar-responsive water-solubilization without any cytotoxicity, to create vascularized channel structures in collagen gels. Gelatin hydrogel rods with the property of sugar-responsive water-solubilization were prepared. Briefly, m-aminophenylboronic acid (APBA) of a sugar-responsive moiety was introduced into gelatin with a weight-average molecular weight of 100,000 and an isoelectric point of 5.0 (Nitta Gelatin Inc., Japan) by using N-hydroxysuccinimide and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide. An aqueous solution of the APBA-introduced gelatin was poured into a polypropylene dish and dried up to obtain APBA-gelatin sheets. Then, the resulting sheet was cut into a rod with a diameter of 300 um and a length of 1 cm. The rod was water-solubilized in a culture media with sorbitol, whereas no water-solubilization was observed in the absence of sorbitol. Since the sorbitol binding allows the boronic acid group to generate negative charges, the resulting electrostatic repulsion could disrupt hydrophobic interaction between the APBA groups for the water-solubilization of the rod. Green fluorescent protein (GFP)-labeled mouse endothelial cells (MS-1) were pre-seeded onto the surface of the rod and the resulting MS-1-attaching rod was embedded in collagen gels. Upon culturing in the culture media with sorbitol, the pre-seeded GFP-labeled MS-1 were found on the channel surface in the collagen gel, probably due to transferring the attached MS-1 to the channel surface simultaneously with the rod removal. As a result, vascularized channel structures were introduced into the collagen gel. Under a flow culture condition, MS-1 survived and proliferated on the wall surface of channel in the collagen gel even after one week of culture. The present hydrogel system of sugar-responsive water-solubilization is a promising sacrificial template to create vascularized channel structures in the collagen gel in vitro.

Session No.: S01-06

Antifouling and Antimicrobial Zwitterionic Nanocomposite Hydrogels as Effective Infected Chronic Wound Dressing

Kang-Ting Huang[1], Pai-Shan Hsieh[2], Yun-Lung Fang[2], Chun-Chang Lee[2], Niann-Tzyy Dai[2], Chun-Jen Huang[3]

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The management for the infected wounds is a critical issue and remains great challenge in the medical care. Generally, an ideal wound dressing should provide a warm moist environment, removal of excess exudate and prevention of harmful bacteria into the wound. In this study, the antimicrobial nanocomposite hydrogel was developed from zwitterionic poly(sulfobetaine acrylamide) (pSBAA) as effective infected chronic wound dressing. The swelling ratio of zwitterionic hydrogel was superior to the non-ionic 2-hydroxyethyl methacrylate (pHEMA) hydrogel in mimic wound exudate. The addition of nanoclay evidently improve the mechanical property of hydrogel. In addition, the anionic charged surface of nanoclay can provide a template to reduce silver nanoparticle. The morphology of silver nanoparticles were observed with transmission electron microscopy (TEM). The cytotoxicity of pSBAA nanocomposite hydrogel was evaluated with NIH-3T3 fibroblast by the MTT assay, the results indicated negligible cytotoxicity after 3 days incubation. In the other hand, the zwitterionic hydrogel showed notable resistance to cell attachment and protein adsorption. The zone of inhibition of pSBAA zwitterionic hydrogel was shown can effectively against gram positive S. epidermidis. For the clinical applications as wound dressing, we created infected diabetic wound on mouse and compared newly developed pSBAA nanocomposite hydrogel with commercially available products. We demonstrated that non-adhesive pSBAA nanocomposite hydrogel enable to be readily removed from the wound. Moreover, the wound recovery results with nanocomposite hydrogel by visual observation showed the complete heal after 15 days. Also, the histological examination confirmed that the zwitterionic hydrogel exhibited thorough reepithelialization and complete formation of new connective tissues in infected diabetic wound after 15 days, which was faster than other commercial dressings. Consequently, we demonstrated that the antimicrobial pSBAA nanocomposite hydrogel can serve as an effective dressing for the wound management. This antimicrobial zwitterionic wound dressing can as promising candidates for healing infected chronic.

Session No.: S02-01 Keynote Speaker

Designing and Controlling Bioactive Molecules for Bone Regeneration in Orthopaedics

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Worldwide, patients continue to suffer from lack of bone. Gold standard treatment is the use of autologous bone graft obtained from the patient. This bone source has a limited quantity and the quality is dependent on the individual patient. Therefore, bone repair by tissue engineering has attracted broad attention. Despite the continuing development of hormones and other bone-stimulating molecules, bone morphogenetic proteins (BMPs) remain the most potent inducers of bone formation. In particular, BMP-2 is widely recognized to be a powerful osteoinductive factor. Endogenous BMP-2 is also important for normal bone homeostasis and is upregulated immediately following bone trauma by recruitment, proliferation and differentiation of osteoprogenitor cells. In the clinical setting, BMP-2 absorbed into a bovine collagen type I sponge in the treatment of degenerative disc disease and fracture non-union but its usefulness has been questioned. BMP-2 efficacy in the clinical setting is remarkably low and delivery of supraphysiological (milligram) doses of the growth factor is needed to obtain therapeutic effects. These high doses are linked to adverse effects. As a result, improved methods of delivery and other methods to stimulate bone growth are being explored. One potentially much more powerful option that has been suggested since some time is to affect target cells on the mRNA level instead of the protein level. However, despite the advances in designing and understanding the role of nucleic acid in several disease states, clinical translation of nucleic acid based therapy has met with limited success. This failure is attributed to our limited possibilities in translocating these useful molecules through the cell membrane and out of the endosome using safe and non-toxic methods. In order to overcome this, we have developed a carrier-free concept that has the potential for therapeutic applications.

Session No.: S02-02 Keynote Speaker

Bone Tissue Engineering and Regenerative Medicine 2.0 - Paradigm Shift from "Proof-of-Concept" to "Proof-of-Value"

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Bone tissue engineering (BTE) strategy provides a promising approach for large bone defect treatment. Despite its first clinical application report in 2001, BTE strategy still stays as a laboratory technique rather than a regular clinical practice, with very limited clinical impact so far. In order to understand the major bottlenecking factors that hinder the fast clinical translation of BTE technology, the development of tissue engineering technology will be compared with computer technology in order to illustrate the influence of “Proof-of-concept (POC)” and “Proof-of-value (POV)” oriented research strategy on the translation of new technology. Generally, it can be regarded as the BTE 1.0 development stage for the past thirty-year’s R&D effort. BTE 1.0 stage is the POC oriented with the goal to prove the scientific feasibility and clinical efficacy and safety of BTE concept. In order to facilitate its wider clinical application and the final translation from a lab technique into a routine clinical therapeutic practice, we believe, in the subsequential BTE 2.0 stage, the focus of our research should be shift from POC to POV, whose mission is to improve and achieve sufficient clinical and commercial value of BTE strategy to replace the current technique. I will discuss and illustrate the Low-Value points of current BTE strategy, such as unavailability off-the-shelf, high cost, complicated manufacturing process and so on and share with you our POV research efforts and thoughts on how to conquer these problems one by one. We believe, similar to the development of computer industry, the next stage of POV-orientated R&D effort (BTE 2.0 stage) will be the most critical and essential stage in order to boost up the final translation of BTE strategy into the real clinical technique, and facilitate the commercialization and maturation of the new industry of tissue engineering and regenerative medicine.

Session No.: S02-07 Invited Speaker

An Osteoconductive and Biodegradable Poly (E-Caprolactone)/Calcium Phosphate Composite Fixator for Osteofixation

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Biodegradable polymer fixators have been used widely in fracture managements, especially in the non-loading bones. However, shortages such as insufficient mechanical strength, inappropriate degradation time, lack of radiolucency, and foreign body reactions during bone remodeling are remaining. In this study, calcium phosphate ceramic (CPC) and poly(ε-caprolactone) (PCL) were used to fabricate biodegradable orthopedic fixation devices. Different weight ratios of CPC were added to PCL, and results revealed that the PCL/CPC composites had good radio-opacity, mechanical properties, and biocompatibility. CPC was transformed into hydroxyapatite when the composites were immersed in simulated body fluid. The PCL/CPC composite had an increasing compressive strength after setting, and this self-reinforcing property contributed to the hydration of CPC and formation of apatite crystals. PCL/CPC composite was then used to prepare bone screws for animal study. In vivo performances of the composite screws were verified in the distal femurs of rabbits. No postoperative mortality or complications were noted in 6 months post-surgery. Biodegradation of the PCL/CPC screws and newly formed bony tissue around the degraded composites were shown on both micro-computed tomography and histology, no peri-implant bone resorption was noted. The self-reinforcing PCL/CPC composite can be used to fabricate biodegradable fixators for fracture fixation.

Session No.: S02-03

Osteogenic Differentiation of Preosteoblasts on a Hemostatic Gelatin Sponge

Zong-Keng Kuo[1], Po-Liang Lai[2], Elsie Khai-Woon Toh[2], Hsiang-Wen Tseng[3], Chih-Chen Chen[4], Chao-Min Cheng[4]

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Bone tissue engineering provides many advantages for repairing skeletal defects. Although many different kinds of biomaterials have been used for bone tissue engineering, safety issues must be considered when using them in a clinical setting. In this study, we examined the effects of using a common clinical item, a hemostatic gelatin sponge, as a scaffold for bone tissue engineering. The use of such a clinically acceptable item may hasten the translational lag from laboratory to clinical studies. We performed both degradation and biocompatibility studies on the hemostatic gelatin sponge, and cultured preosteoblasts within the sponge scaffold to demonstrate its osteogenic differentiation potential. In degradation assays, the hemostatic gelatin sponge demonstrated good stability after being immersed in ddH2O for 8 weeks (losing only about 10% of its net weight), but pepsin and collagenases readily biodegraded it. The hemostatic gelatin sponge demonstrated good biocompatibility to preosteoblasts as demonstrated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay, confocal microscopy, and scanning electron microscopy. Furthermore, osteogenic differentiation and the migration of preosteoblasts, elevated alkaline phosphatase activity, and in vitro mineralization were observed within the scaffold structure. Each of these results indicate that the hemostatic gelatin sponge is a suitable scaffold for bone tissue engineering.

Session No.: S02-04

The Exosomes from Adipose Tissue-derived Mesenchymal Stem Cells: Potential Application for Bone Tissue Regeneration

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Mesenchymal stem cells (MSCs) are a promising cell source for tissue repair and regeneration. However, direct MSCs transplantation to tissue injury sites has its inherent drawbacks such as senescence-induced genetic instability and limited cell survival. The aim of this study was to employ the exosomes produced by MSCs for use in bone tissue regeneration. We demonstrated that adipose tissue-derived MSC (ASCs)-derived exosomes (ASC-EXO) promoted the proliferation, mobilization and osteogenic differentiation of human primary osteoblastic cells (HOBs). We further established that the trophic effects of ASC-EXO on HOBs were potentiated when ASCs were pre-conditioned with tumor necrosis factor-alpha (TNF-α) for 3 days, mimicking the inflammatory phase of bone fracture healing. In addition, we showed that TNF-α pre-conditioning significantly increased the Wnt-3a content ASC-EXO, and blocking Wnt signaling inhibited the osteogenic gene expression levels in HOBs cultured in the conditioned medium collected from TNF-α pre-conditioned ASCs. In summary, this study demonstrates that ASC-derived exosomes are capable of promoting proliferation, migration, and osteogenic differentiation in HOBs, and the effects are further harnessed by TNF-α pre-conditioning through wnt signaling pathway, suggesting that ASC-derived exosomes might offer a promising approach to replace direct stem cell transplantation for bone repair and regeneration.

Session No.: S02-05

Osteogenic Ability and Cell Survival of Mesenchymal Stem Cells Seeded on Coral Scaffolds and Implanted in Either Ectopic or Orthotopic Sites in Mice

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Tissue constructs containing mesenchymal stem cells (MSCs) are appealing strategies for repairing large segmental bone defects but they do not allow consistent bone healing and early cell death was identified as a cause of failure. However, little is known about cell survival in the clinical micro-environment encountered during bone healing process. Osteoconductive coral scaffold with or without luciferase-labelled human MSCs were implanted either in a critical-segmental-femoral-bone defect stabilized by plate or subcutaneously in 44 mice. Cell survival was evaluated by serial bioluminescence imaging (BLI) and osteogenic capabilities by histology and microCT. Comparisons between groups were performed with two-way-ANOVA test. Twenty mice were sacrificed 2 weeks after surgery for "short term" evaluation and 24 mice at 10 weeks for "long term" evaluation. BLI provided evidence of fast and continuous cell death: 85% decrease of the BLI signal over the first 2 weeks in both location; in fact, less than 2% of the initial cell number were present in all constructs analyzed 4 weeks post-implantation and less than 1% of the initial cell number by 8 weeks post-implantation. By 2 weeks post implantation, the amount of newly formed bone was self-limited and was similar between ectopic and orthotopic group. By 10 weeks post implantation, bone formation was significantly enhanced in the presence of MSCs in orthotopic site and the amount of newly formed bone in cell-containing constructs implanted in orthotopic locations was significantly higher than that observed in the ectopic group. Our results indicated that hMSCs promote bone formation despite early and massive cell death when loaded on coral scaffolds. Interestingly, bone formation was higher in orthotopic than ectopic site despite a same survival pattern. Ectopic implantation of cell-containing constructs is suitable to evaluate cell survival, but assessment of bone formation ability requires orthotopic implantation.

Session No.: S02-06

3D Plotting of Highly Uniform Sr5(Po4)2Sio4 Bioceramic Scaffolds for Bone Tissue Engineering

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Bioceramics are playing an important role in the regeneration of large bone defects. However, it is challenging to design bioceramic scaffolds with proper ionic components and beneficial osteo/angio-stimulation ability for bone regeneration application. In this study, we successfully synthesized a pure-phase Sr5(PO4)2SiO4 (SPS) bioactive ceramic through the solid-state reaction method and further prepared highly uniform SPS bioceramic scaffolds with controlled macropore size and mechanical strength by 3D-Plotting technique, and the biological responses of rabbit bone marrow stromal cells (rBMSCs) and human umbilical vein endothelial cells (HUVECs) after cultured with different concentrations of SPS extracts and porous scaffolds were systematically studied. The results showed that the ionic products from SPS bioceramics significantly stimulated the proliferation, alkaline phosphate (ALP) activity and osteogenesis-related gene expression (Runx2, ALP, OCN, OPN) of rBMSCs as well as the proliferation and angiogenesis-related gene expression (VEGF, KDR, eNOS, HIF 1α) of HUVECs. 3D-Plotted SPS scaffolds could effectively support the attachment and proliferation of both rBMSCs and HUVECs, and the proliferation rates of two kinds of cells in SPS scaffolds were distinctively higher than those in β-tricalcium phosphate (β-TCP) scaffolds prepared by the same method. In addition, the compressive strength of SPS scaffolds could be well controlled in the range of 8~30 MPa when their pore size varied from 100 to 300 μm, which was significantly higher than those of β-TCP scaffolds with similar pore size (~1.5 times). Our results demonstrated that 3D-Plotted SPS bioceramic scaffolds with such a specific ionic combination and high mechanical strength as well as good degradability possessed the ability to stimulate both osteogenic and angiogenic differentiation of tissue cells, indicating that they might be a promising biomaterial for bone tissue engineering.

Session No.: S03-01 Keynote Speaker

Peripheral Nerve Regeneration by Using Various Biomaterials

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Objectives : The search for a suitable material to guide the nerve regeneration has been a challenging tasks. Many kinds of materials including artificial or natural products has been studied for this purpose, despite its limitations and complications. We reviewed the current trends of investigation using various natural, artificial or combined biomaterials. This presentation include the results of our research for peripheral nerve regeneration using conduit consisting with porcine small intestinal submucosa(SIS), PLCL (combination of PGA and poly-caprolactone) and PLCL-SIS combination materials. Material and Methods : All experiments were done with rat sciatic nerve trans-section model. PLCL and PLCL combination nerve conduit were made with 3D printing technology. Silicone was used as control. Motor function test, electromyography study and histologic analysis were done for evaluating the results. Results : SIS conduit ! was good for the regeneration of peripheral nerve regeneration, but it was too weak for long segment nerve graft. PLCL conduit was easy to make but less regeneration activity and more inflammation. PLCL-SIS combination conduit was good for regeneration and less inflammation. 3D printing technology could give more effective structure for regeneration activity of conduit Conclusion : These results show that PLCL-SIS conduit was good option as a natural-synthetic combined biomaterial for peripheral nerve regeneration. And more, it could be used as biocompatible barriers covering neural tissue, for example, dural repair, since this material contains many kinds of nerve growth factors.

Session No.: S03-02 Invited Speaker

The Effect of Chitosan-siloxane Porous Hybrids on Nerve Regeneration and Functional Recovery

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Peripheral nerve injuries have a high incidence in today's society [1]. Despite recent progress in peripheral nerve trauma management, recovery of functional parameters is usually far from normal and thus much attention is being paid to nerve regeneration research. Over the last years, the research on entubulation nerve repair has developed along two main lines: (1) testing different biomaterials for fashioning of the conduit; (2) testing different additives for enriching the nerve guide. As regards the first research line, many properties are desirable for a nerve scaffold. They included: (a) permeability to prevent fibrous scar tissue invasion allowing nutrient and oxygen supply; (b) mechanical strength to maintain a stable support structure for the nerve regeneration; (c) immunological inertness with surrounding tissue; (d) biodegradability to prevent chronic inflammatory response and pain due to nerve compression; (e) easy regulation of conduit diameter and wall thickness; (f) surgical amenability. Among the various biomaterials proposed for the fashioning of nerve conduits, chitosan has recently attracted much attention because of its biocompatibility, biodegradability, low toxicity, low cost, enhancement of wound-healing, and antibacterial effects [2]. We have tried the nerve regeneration using flexible and biodegradable chitosan-γ-glycidoxypropyltrimethoxysilane (GPTMS) porous hybrids [3-5]. In this study, we show some results about the nerve regeneration and the functional recovery using the hybrids.

Session No.: S03-03 Invited Speaker

Multi-channeled Gelatin Scaffold Incorporated with Neurotrophic Gradient and Nanotopography as Nerve Guidance Conduit for Peripheral Nerve Regeneration

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Artificial nerve conduits can be used as nerve grafts to enhance the regeneration of large nerve defects. Many studies suggest that fibrous and multi-channeled scaffolds can potentially be used for nerve regeneration, as the aligned fibers can provide the guidance effect for axonal growth and the mulit-channeled structure mimics the fascicular architecture and decreases the nerve dispersion. A number of studies indicate that concentration gradients of guidance molecules influence axonal growth and cell migration. However, up to now, few studies have combined aligned fibers, mulit-channeled structure and neurotrophic concentration gradients in one integrated system to study their synergistic effect on peripheral nerve regeneration. In this study, aligned fibers were fabricated using an electrospinning technique. The multi-channeled scaffold incorporated with concentration gradient of neurotrophic growth factors including NGF and BDNF was created by a gradient maker. We expect that (i) the aligned fibers can guide the axonal growth to particular direction; (ii) mulit-channeled structure can provide necessary support and decrease the nerve dispersion; (iii) the guidance of molecules gradient can promote axon outgrowth from the proximal stump to distal end.

Session No.: S03-04

Conveniently Templated Porous Chitosan Conduit with Highly Aligned Luminal Wall and Seamless Side Wall for Peripheral Nerve Regeneration

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[1]Nantong University

In this study, we developed a facile and novel one-step lyophilization process for fabricating the side wall seamless nerve conduits, with the longitudinally aligned structure on luminal wall while porous structure on middle wall. The prepared conduit then was used to bridge a 10 mm rat sciatic nerve gap. The porous chitosan conduit with ridge/groove structure on luminal wall was fabricated by lyophilization method via a cylindrical polydimethylsiloxane (PDMS) stamp. The morphology, porosity and mechanical properties were measured. The effect of the prepared conduits on 10-mm nerve gap in rat sciatic nerve was evaluated. A series of behavioral techniques were used to assess the physiological recovery of the animals over a period of 1-12 weeks after the initial surgery. Finally, the axonal regeneration of the injured nerve in the proximal and the distal ends was quantified via morphometrics evaluation. The results indicated that the porous chitosan conduit with ridge/groove structure on luminal wall was successfully fabricated. The conduit displayed consistent tensile behavior with the normal nerve tissue. During 1–2 weeks after nerve grafting in rat sciatic nerve,the lunimal micropatterned chitosan conduit significantly accelerated axonal growth, showing a positive reaction to S-100 (a Schwann cell marker). At 12 weeks after nervegrafting,the results of histological and functional assessments showed that the lunimal micropatterned chitosan conduit yielded an improved outcome of functional recovery and nerve regeneration, which was superior to the control conduits without luminal micropatterning and displayed comparable therapeutic effects to autografts. In summary, the porous chitosan conduit with ridge/groove structure on luminal wall could significantly promote peripheral nerve regeneration in vivo. The proposed micropatterned conduits may be exploited to imitate various tubular tissues, including nerve, tendon and vascular, etc, which will show potentially huge applications in tissue engineering and regenerative medicine.

Session No.: S03-05

Bridging Peripheral Nerve Defects with a Tissue Engineered Nerve Graft Composed of an in Vitro Cultured Nerve Equivalent and a Silk Fibroin-based Scaffold

Xin Tang[1]

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Tissue engineered nerve grafts are considered as a promising alternative to autologous nerve grafts used for peripheral nerve repair. The differences between these two types of nerve grafts are mainly in the regenerative microenvironment established by them. To construct ideal tissue engineered nerve grafts, it is therefore required to develop a better way to introduce biochemical cues into a neural scaffold, as compared to single or combined use of support cells and growth factors. Here, we used a co-culture system of dorsal root ganglia and Schwann cells to create an in vitro formed nerve equivalent, which was introduced into a silk fibroin-based scaffold to furnish a tissue engineered nerve graft (TENG). At 4- and 12- weeks after the TENG was implanted to bridge a 10-mm-long sciatic nerve defect in rats, histological and functional assessments as well as Western blot analysis were performed to evaluate the influences of the TENG on peripheral nerve regeneration. We found that at an early stage of nerve regeneration, the TENG significantly accelerated axonal growth, and up-regulated expressions of N-cadherin and PMP22. Twelve weeks after nerve grafting, the TENG produced a further improved outcome of nerve regeneration and functional recovery, which was more close to that of the autologous nerve graft than that of the silk fibroin-based scaffold. The introduction of an in vitro cultured nerve equivalent into a scaffold might contribute to establishing a native-like microenvironment for nerve regeneration.

Session No.: S03-06

Peptide Gradient Along Micropatterned Surface of Poly(L-lactide-co-caprolactone) Conduit for Peripheral Nerve Repair

Deteng Zhang[1], Shan Yu[1], Changyou Gao[1]

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Nerve guidance conduit (NGC) for peripheral nerve repair has been demonstrated effective to guide the out growth of amputated nerves and to resist the abnormal growth the surrounding tissues into the conduit. However, there are still lack of effective methods to directly guide and continuously stimulate Schwann cells proliferation and migration, and to induce the axons to bridge the damaged proximal and distal stumps orientedly[1]. Here, we developed a new method to prepare NGCs by combining gradient peptide with stripe pattern on biodegradable poly(L-lactide-co-caprolactone) (PLCL) conduit, following by reeling and sealing of the membrane. The groove and ridge with a 40*20μm feature were introduced on the surface of PLCL film by a template thermo-pressing method. Along the stripes, amino group density gradient was prepared through gradient aminolysis[2], which was transferred into CQAASIKVAV density gradient by covalent linking with glutaraldehyde. The resulted peptide density gradient ranged from 0.38 to 1.29 μg/cm2 on the PLCL membrane. The adhesion, orientation and migration of Schwann cells and PC12 axonal alignment growth were studied in vitro. The double functional NGCs with physical and chemical cues are expected to interplay together to achieve better peripheral nerve regeneration in vivo. Acknowledgement: Financial support by Natural Science Foundation of China (21434006, 21374097) References [1] Jacob L. Roam, Donald L. Elberta, et al., Biomaterials, 2015; 72: 112-124 [2] Yang Zhu, Changyou Gao, et al., Biomacromolecules, 2013; 14: 342-349

Session No.: S04-01 Keynote Speaker

Tendon Engineering: from Cell-based Approach to Inductive Material Based Cell-free Approach

Wei Liu[1], Wenbo Wang[1], Yuan Shi[1], Xunxun Lin[1], Dan Deng[1], Bin Wang[1]

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Cell, scaffold and tissue regeneration environment are the major three components of traditional tissue engineering approach. Cell based approach may limit its application only to individual based therapy, whereas cell-free scaffold approach allow for product based therapy. Recent studies indicate that integration of tissue specific topographical structure and extracellular matrix into scaffold fabrication is possible to generate tissue inductive scaffold for in situ tissue regeneration. Our previous studies indicated that seeded cells of engineered tendon once implanted in vivo, are likely to be replaced with host cells during tissue remodeling process. In recent studies, we also showed that simulation of tendon topographical structure in scaffold fabrication was able to induce fibroblast transdifferentiation into tenocyte phenotype and also facilitate recruiting of host cells into the scaffold for tendon regeneration. Furthermore, with combination of hyaluronic acid modification and mechanical loading, the cell-free scaffold could well regenerate tendon tissue in a rabbit Achilles tendon repair model. This talk introduce our related research work to demonstrate the transition from cell-based to cell-free approaches in tendon regeneration.

Session No.: S04-02 Invited Speaker

Biomimetic Tendon Matrix Composite Gradients for the Acl Ligament-to-bone Junction Reconstruction

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The ligament/tendon-to-bone-junction healing still represents a great challenge today, mainly because of the relative avascularity and multi-tissue transition structure of the junction. Attempts such as using stem cells, bioactive factors and synthetic materials, have fallen short of recapitulating the complex structure-function relationships in native tissue interfaces.
Extracellular environment surrounding cells influence cell morphology, adhesion, proliferation, differentiation. A number of biomimetic scaffolds have been developed with specific physical patterns to control cell behavior, which have been proved have the similar effects as the chemical cues. Our previous studies showed that aligned nanofiber promotes the teno-lineage differentiation of stem cells and the random nanofiber promotes the osteo-lineage differentiation both in vitro and in vivo. These studies indicate the possibility to modify the biomaterials by integration of topographic cues for ligament to bone regeneration.

Therefore, in this study, firstly, we explored easy and high efficient methods of ligament decellularization. Secondly, we developed a biomimetic tendon-to-bone ECM composite that with distinct yet continuous aligned and random pattern on the surface of the decellularized tendon ECM. And then we performed an in vitro assessment of the differentiation of the BMSCs on the composite and the in vivo evaluation of the ligament-to-bone reconstruction in a rabbit model of ACL defect.

The chondrogenesis and osteogenesis capability of the rabbit mesenchymal stem cells on the modified ECM were tested in vitro. The results showed that the random tendon ECM promotes higher chondrogenesis and osteogenesis-related gene expression and exhibits higher osteogenic-induced capability than the untreated tendon ECM in vitro. Importantly, in the rabbit anterior crucial ligament (ACL) reconstruction model in vivo, micro-computed tomography (Micro-CT) and histological analysis showed that the modified random-aligned-random composite enhances the bone and fibrocartilage formation in the interface and is more efficiently than the unmodified tendon ECM. Therefore, this results demonstrated

Session No.: S04-03

Cell Therapies for Tendon Surgery

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Tendons and ligaments are frequently damaged during rigorous activities such as sport or the process of aging. Despite their relatively high prevalence and morbidity of tendon and ligament injury in sport, most treatments have been proven to provide no, or only modest short-term benefits. Traditional first-line treatments may provide modest short-term benefits for pain. Current surgical and therapeutic treatment options include prosthetic devices, autografts, allografts, or xenografts, however these exhibit only limited success. Over the past 15 years, we focus exclusively on the translational medicine program of tendon repair and regeneration. We have observed that depletion of the functional tenocyte pool in the region of the tear may account for fatigue of the normal healing response. On the basis of the pathology studies and pre-clinical animal work, we have developed cell therapy strategies for tendon and ligament repair in human. These include the development of ultrasound guided autologous tendon cell injection (ATI) for tendon tear and the Matrix argument autologous tendon cell implantation (MATT) for surgical repair of tendon and ligament. To date, a series of clinical trials on ATI were conducted in patients with different anatomical sites of tendinopathy including chronic lateral epicondylitis, rotator cuff tendon tear, gluteal tendinopathy. We have showed that ATI, the first homologous cell therapy technique developed for the treatment of tendinopathy, has the potential to address this unmet clinical need by replenishing the pool of functional tenocytes in the site of tendinopathy. We have also developed sensor controlled bioreactor for the generation of neo-tendon tissue using autologous tendon cells from a needle biopsy and collagen based ligament for ACL reconstruction. Here we will provide an overview of the translational program of tendon and ligament regeneration with a strong focus on the clinical data of ATI, MATT and human neo-tendon implant.

Session No.: S04-04

Assessment of in Vivo Cellularization of a Novel Ligament-substitute "Decellularized Xenograft" Derived from Bovine Extensor Digitorum Tendon in a Rat Model of Anterior Cruciate Ligament Reconstruction

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Anterior cruciate ligament reconstruction (ACL-R) using autograft is a standard method. However, in addition to donor site morbidity, autografts are prone to be insufficient in quantity in case of revision or multi-ligament reconstruction surgery. There are no reports that allografts or artificial grafts have superior performance to autografts. Development of more biocompatible graft is expected in order not to sacrifice healthy tissue. The purpose of this study was to assess in-vivo cellularization of a decellularized bovine xenograft in a rat ACL-R model. Bovine extensor digitorum tendon was decellularized using 1wt% of deoxycholic acid under a pulsatile flow and pressure circulation in parallel with a microwave irradiation. Eighty-four 12-week-old male SD rats (350±50 g) were used. In forty-two rats, the right knee underwent ACL-R using decellularized xenografts (group-D). In the other forty-two rats, the right knee underwent ACL-R using autologous Achilles tendon (group-A). The size of graft was about φ1 mm × 10 mm. In both group, six each were sacrificed at 1,2,4,8,16,26,52 weeks for histological evaluation. In group-D, decellularized tendons were not rejected and in-vivo cellularized with autologous cells. At 16 weeks after implantation, cell densities in group-D were reached those of native ACL, and kept plateau at 26 and 52 weeks. In group-A, the autologous tendons became acellular in vivo in 2-4 weeks, then, were gradually recellularized. The cell densities in group-A were comparable to those of native ACL of rats at 26 weeks, and kept plateau at 52 weeks. Faster recellularization was observed in the decellularized xenogafts than autografts.

Session No.: S04-05

Functional Tissue Engineering of Tendon by Bone Marrow Derived Mesenchymal Stem Cells

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Introduction Tendon injuries are common and present a clinical challenge to orthopedic surgery mainly because these injuries often respond poorly to treatment and often resulting in long-term impairment. The inferior natural healing responses are attributed mainly to insufficient or failed tenogenesis. The focus of this study is to investigate the growth factors that control tenogenesis of one marrow derived mesenchymal stem cells (BMSCs) in order to optimize tissue engineering techniques for treating tendon disorders. • Subjects and Methods We chose three representative factors from current reported tenogenic factors. We compared the tenogenic efficiency of these three factors (CTGF, GDF-7 and TGF-ß1) in parallel and establish an efficient inductive approach to induce tenogenesis of mesenchymal stem cells. • Results Based on comparative studies of TGF-ß1, BMP12, CTGF and their combinations on tenogenic induction of BMSCs, it was found that TGF-ß1 alone induced teno-lineage specific gene scleraxis expression and collagen production significantly and efficiently. In addition, TGF-ß1 combined with CTGF elevated tenomodulin mRNA and protein expression at day 7. Hence, the stepwise tenogenic differentiation approach was established by first using TGF-ß1 stimulation for 3 days followed by combination with CTGF for another 7 days. In ectopic tendon regeneration model and in-situ rat patellar tendon repair, neo-tendon formed by induced BMSCs had much better structural and mechanical properties than did controls, as evidenced by histological analysis, collagen I and tenomodulin immunohistochemical staining data, as well as mechanical tests. • Discussion and Conclusion The study demonstrates that induction of BMSCs using growth factors through stepwise differentiation enhances tendon formation and repair. This effective tenogenic approach will not only enhance the effectiveness of the cell therapy in treating tendon disorders, but also can be served as a platform for underlying molecular mechanism research.

Session No.: S04-06

Soft Tissue Flap Engineering with Pre-vascularized Extracellular Matrix Scaffold

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INTRODUCTION: Revascularization of the engineered biomaterial with host vasculature is important for successful implantation. This study aimed to develop pre-vascularized flap bioscaffold to improve the vascularization and survival of engineered tissue after transplantation [1, 2]. METHODS: A perfusion-decellularization protocol was developed to remove cell components from pig musculofascial flap (DMF) and rat skin/adipose flap (DSAF) [3]. hASCs were integrated with DMF and DSAF to test their biocompatibility. DMF and DSAF were implanted in Fisher rats to evaluate implant-host reaction. Re-cellularized and pre-vascularized DMF and DSAF with hASCs and HUVECs were then transplanted as free flap in a nude rat model. RESULTS: H&E, DAPI staining and DNA quantification confirmed cell removal in DMF and DSAF. DMF and DSAF maintained natural extracellular matrix structure with 3D nanofibrous features (SEM imagining), strong mechanical properties, biochemical compositions (collagen+, laminin+, MHC1- e.g.) (IHC staining and Mass Spectrometry), and microcirculatory network with the dominant vascular pedicle structure. DMF and DSAF provide a niche for hASCs and HUVECs proliferation. DMF and DSAF caused little foreign body response in vivo (few CD4+ / CD8+; M1-/M2+). Re-cellularized flap constructs were successfully transplanted by microsurgery anastomosis pedicle with recipient femoral artery. Implanted engineered flap was fully survived and remodeled as indicated by pedicle patency and soft tissue formation at 3 months. CONCLUSIONS: Pre-vascularized decellularized flap matrix provides novel promising composite bioscaffold for large-scale soft tissue engineering and reconstruction. REFERENCES: [1] J. Rouwkema, N. Rivron, Van Blitterswijk (2008). Vascularization in tissue engineering. Trends Biotechnol. 26:434-41. [2] M. Lovett, K. Lee, A. Edwards, D. Kaplan (2009). Vascularization strategies for tissue engineering. Tissue Eng Part B Rev. 15:353-70. [3] L. Wang, J. Johnson, D. Chang, Q. Zhang (2013). Decellularized musculofascial extracellular matrix for tissue engineering. Biomaterials. 34:2641-54. ACKNOWLEDGEMENTS: This work was supported in part by Plastic Surgery Foundation (to Q. Zhang.)

Session No.: S04-07

The Application of Aligned Electrospun Nano-/Micro-fibrous Membrane as Mesenchymal Stem Cell Culture Matrix for Artificial Tendons Reconstruction

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Ruptured Achilles tendons account for over 50% of all sports-related injuries, leading to a high demand for tendon reconstruction procedures as there is no standard procedure for tendon repair and conventional tendon repairs require great technical skill. Biocompatible and biodegradable polymers poly-L-lactic acid (PLLA) and polybutylene succinate (PBS) were used to fabricate both random and highly aligned scaffolds through electrospinning to provide a extracellular matrix-mimicking environment suitable for the growth of mesenchymal stem cells and primary tenocytes with fiber diameters ranging from approximately 500 nanometers to up to 20 micrometers measured under a stereo microscope. Membrane thickness, fiber diameter, and porosity were characterized and the environmental cues of the membranes on cell morphology were investigated using fluorescent mouse muscle fibroblast cells cultured in DMEM medium at 37oC under a CO2 atmosphere and examined every two days over the course of 2 weeks showing parallel and highly aligned cell growth on the aligned scaffolds in contrast to disordered cell growth on the random scaffolds. Cell proliferation of rabbit tenocytes and scaffold cytotoxicity of NIH 3T3 fibroblast cells on random and aligned electrospun scaffolds were tested using WST-1 assay. Tensile loading tests will be performed for random and aligned on random electrospun scaffolds from different polymers to compare the effects of different materials and fiber structure on tensile strengths. Ultimately, primary tenocytes and bone marrow derived mesenchymal stem cells will be cultured on layered aligned and random electrospun membranes to form an artificial tendon graft for clinical use in reconstruction procedures related to tendon injuries.

Session No.: S05-01 Keynote Speaker

Design and Preparation of Hybrid and Biomimetic Porous Scaffolds for Tissue Engineering

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[1]National Institute for Materials Science

Porous scaffolds of biodegradable polymers have been broadly used in tissue engineering because of their versatile properties of biodegradation, good biocompatibility and easy formation into different porous structures. They can be prepared by different methods to control their microporous structures and mechanical properties. We have designed and prepared a few types of functional porous scaffolds from biodegradable synthetic polymers, naturally derived polymers and cultured cell-derived matrices. The first one is 3D stepwise chondrogenesis-mimicking ECM scaffolds that were prepared from mesenchymal stem cells (MSCs) by controlling the stages of chondrogenic differentiation. ECM scaffolds mimicking the early stage and late stage of chondrogenesis were obtained when MSCs were cultured in the chondrogenic medium for 1 and 3 w, respectively. The ECM scaffolds had different compositions as shown by immunohistochemical analysis. The three ECM scaffolds had different effects on chondrogenesis of MSCs. The CE-ECM scaffold facilitated chondrogenesis, however the CL-ECM scaffolds remarkably inhibited chondrogenesis of MSCs. The second one is porous scaffolds prepared by using pre-prepared ice particulates as a porogen material. Scaffolds prepared with ice particulates showed well interconnected spherical pore structures. The scaffolds facilitated an even cell distribution throughout the scaffolds and the regeneration of cartilage tissue when chondrocytes were cultured in the scaffolds. The third one is hybrid scaffolds of synthetic polymers and naturally-derived polymers. Collagen sponge or microsponges were introduced in the pores or openings of mechanically strong synthetic polymer skeletons to construct the hybrid structures. The skeletons of synthetic polymers provided high mechanical strength, while the collagen sponge and mircosponges facilitated cell seeding and distribution. The hybrid polymer scaffolds were used for three-dimensional culture of fibroblasts, chondrocytes and bone marrow-derived MSCs for tissue engineering of dermal tissue, cartilage and bone.

Session No.: S05-02 Invited Speaker

Soft Porous Scaffold Also Promotes Osteogenesis in a Rat Cranial Defect Model

Chao Zhang[1], Chuntao Liu[1], Xiaoreng Feng[2], Chaowen Lin[2], Chaozhu Chen[1], Bin Chen[2]

[1]Sun Yat-Sen University
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It has been widely recognized that the stiffness of the matrix regulates the differentiation of stem cells, and stiffer matrix would more likely lead to osteogenesis. Three dimensional scaffolds with interconnective pores have shown to be promising candidate material in tissue engineering, and the poroues structure may also play a vital role in the differentiaion of stem cells. In this work, RGD peptide modified poly(ethylene glycol)-co-2-hydroxyethyl methacrylate cryogels with varied stiffness were prepared, and the effect of stiffness and porous structure of the cryogel scaffold on the osteogenesis was studied both in vitro and in vivo. Hard cryogel was found to better promote the the osteogenic differentiation of rMSCs in vitro, as evidenced by immunofluorescent staining of F-actin/vinculin, ALP activity, expression of relative osteogenic gene marker (ALP, Runx2, OCN), and protein expression of OCN by immunohistochemical staining. However, both the hard and soft cryogels facilitated the osteogenesis in a critical-sized cranial defect rat model, as evidenced by the formation of dense bone minerals, dense collagen networks, and osteoid tissue and vessels. It was quite suprising that soft cryogel promoted obvious osteogenesis and remarkable angiogenesis in bone defect due to the adsorption of large amount of osteogenic growth factor (BMP-2) and angiogenesis growth factor (VEGF). Such finding reveals that in addition to the matrix stiffness, the adsorption, entrapment, and concentration of BMP-2 and VEGF in the matices also contribute to the osteogenesis and angiogenesis in vivo. Further understanding of the synergistic effect of stiffness and porous structure of the cryogels on osteogenesis in vitro and in vivo may pave the way for the successful design of scaffolding materias in bone regeneration.

Session No.: S05-03 Invited Speaker

Injectable Cell Scaffold of Hybrid-microsphere for Angiogenesis Therapy

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Cell therapy for angiogenesis aims to improve limb perfusion by enhancing neovascularization using autologous bone marrow mononuclear cells (BMNCs), stem cells and so on. The mechanizum for neovascularization by cell therapy depends on regulation of the secration of proangiogenic factors and endotherial differentiation. The clinical efficiency of cell-based therapeutic angiogenesis in diabetic patients with peripheral arterial disease (PAD), unfortunately, has not been satisfied. Because approximately 80% of the transplanted cells were setimated to disappear from the injection site within 1 week after transplantation. Thus, it is necessary for maintaining transplanted cells in place in order to enhance the therapeutic efficiency. In this report, injectable cell schaffold (ICS), that was hydroxyapatite (HAp) nanoparticles coated bioabsorbable poly(L-lactide-co-caprolactone) (PLCL), was developed [1] and evaluated the efectiveness for angiogenesis therapy. The ICS was fabricated by an o/w-type Pickering emulsion. HAp nanpartices were employed as a particulate emulsifier. The ICS were examined as an injectable cell scaffold for cell-based therapeutic angiogenesis in mice ischemic hind-limbs. After co-injection of BMNCs with the ICS, avoidance rate of limb necrosis statistically increased at three times than that of only BMNCs injection [2]. Therefore, the co-injection of BMNCs with the ICS is very effective for angiogenesis therapy. The therapeutic efficency for the co-injection in diabetic mice with hindlimb ischemia was higher than that of the injection of BMNCs only [3]. The Preparation of pourous nanocomposite microspheres by water-in-oil-in-water (W/O/W) multiple emulsion stablized with HAp nanoparticles was also reported [4]. References: 1. X. Liu, et al., Acta Biomater., 7, 821-828(2011). 2. Y. Miwa, et al., PLoS ONE, 7(e35199), 1-12(2012). 3. K. Takeda, et al., Biochem. Biophys. Res. Commun., 454, 199-124(2014). 4. H. Maeda, et al., Langumuir, 26, 13727-13731(2010).

Session No.: S05-04 Invited Speaker

Hybrid Scaffolds with Bioactive Silicate Components to Stimulate Osteogenic and Angiogenic Differentiations

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The massive bone defects due to traumatic injuries, cancer, and congenital defects, require the use of large bone grafts. It has been shown that implantation of large bone grafts without adequate vascularity usually results in apoptosis and cartilage formation. Therefore, it is considered that both sufficient angiogenesis and osteogenesis is necessary to regenerate the massive defects. A tissue-engineered graft made of novel bioactive scaffolds with mesenchymal stem cells (MSCs) is proposed as a potential bone graft for massive bone defects, due to its improvement on angiogenesis and osteogenesis in vivo. As a key factor, biomaterial scaffolds play an important role in bone tissue engineering. The development of the bioactive scaffolds with excellent osteogenesis and angiogenesis properties is critical for bone regeneration and bone tissue engineering applications. The studies suggest that the functional silicon (Si) element plays an important role in the growth and development of normal bone, cartilage and connective tissue, especially at the early bone growth stages. Herein, we report our progress in the fabrications and properties of the hybrid scaffolds with bioactive silicate bioceramics and bioglass component. The results revealed that the incorporation of the silicate component can apparently enhance the osteogenic and angiogenic differentiations of the mesenchymal stem cells, and stimulate the bone regeneration ability of Poly-D,L-Lactide-Glycolide and silk fibroin materials, and the calcium phosphate based bioceramics. In addition, the mechanisms behind the osteogenesis and angiogenesis of the silicate component were preliminary revealed.

Session No.: S05-05 Invited Speaker

Biofabrication of Biomimetic Composite and Hydrogel Constructs for Tissue Engineering

Wojciech Swieszkowski[1], Marco Constantini[2], Joanna Idaszek[1], Alicja Kosik[1], Nehar Celikkin[1], Jan Brichmann[3], Krzysztof Jan Kurzydlowski[1]

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There is a growing demand for developing new cell-based therapies for tissues repair and regeneration [1]. 3D bioprinting is a fast-emerging technique in tissue engineering which allows for the controlled and simultaneous 3D deposition of living cells and supporting biomaterials [2]. This technique combines the features of 3D printing of the patient-specific scaffolds with the possibility of precisely depositing living cells in the 3D space.

The aim of the study is to demonstrate how to fabricate 3D biomimetic constructs for tissue engineering with high cell density, high cell viability and high printing resolution using a novel 3D biofabrication approach. We used an innovative 3D deposition system based on a coaxial-needles extruder developed in-house. The bioinks were composed of modified biopolymers like gelatin, alginate, hyaluronic acid, chitosan or composites. By changing ink composition, its physicochemical properties could be modulated. For instance, addition of short nanofibers to hydrogel matrix increased its stiffness scientifically. The biomimetic inks were loaded with different types of cell including bone marrow-derived human mesenchymal stem cells or chondrocytes. Using the developed biofabrication method and novel bioinks it was possible to obtain bioactive constructs with cell viability exceeding 90%.

Our findings show that the presented 3D printing method is highly robust and accurate and allows for formation of 3D biostructures which after some period develop into neo-tissues.

References

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Session No.: S05-06

Biomimetic Alginate-chondroitin Sulfate Hybrid Hydrogel for 3D in Vitro Tumor Metastasis Model

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Tumor metastasis with resistance to anticancer therapies is the main cause of death in cancer patients. The molecular mechanisms underlying tumor metastasis are still not well known, and chemotherapy has a limited impact on improving the survival rate. Therefore, it is necessary to develop reliable 3D in vitro tumor metastasis models that can closely recapitulate the pathophysiological features of the native tumor tissue. Our previous work found that the metastatic ability of tumor cells cultured in alginate hydrogel beads (ALG) was significantly higher than 2D-cultured cells. However, bio-inert alginate hydrogel lacks sufficient bioactivity for tumor microenvironment mimicry. It is reported that the expression of chondroitin sulfate (CS) is significantly increased within the ECM of many human solid tumors. In this study, alginate-CS hybrid hydrogel beads (ALG-CS) are developed to mimic the in vivo tumor microenvironment with an abnormally increased expression of CS for the promotion of tumor cell metastasis. It is found that CS molecules can be cross-linked to alginate molecules via coordination of calcium ions when ALG-CS is formed, which enables ALG-CS to possess significantly different physical characteristics, including swelling degree, mechanical stability and permeability, than the traditional alginate beads (ALG). And quantum chemistry calculations show that in addition to the traditional egg-box structure, novel asymmetric egg-box-like structures based on the interaction between these two kinds of polymers are also formed within ALG-CS. The viability of tumor cells was found significantly higher in ALG-CS compared with that in ALG. Moreover, tumor cell metastasis is also significantly enhanced in ALG-CS, as confirmed by the increased expression of MMP genes and proteins and greater in vitro invasion ability. Therefore, ALG-CS could be a convenient and effective 3D biomimetic scaffold that would be used to construct a standardized 3D in vitro tumor metastasis model for tumor research and anticancer drug screening.

Session No.: S06-01 Keynote Speaker

The Injectable Oxidated Hyaluronic Acid/Adipic Acid Dihydrazide Hydrogel as a Vitreous Substitute

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[1]National Health Research Institute

Human vitreous is a gelatinous substance that is predominantly composed of collagen fibril, hyaluronic acid (HA) and water (97–99%). Vitreous substitutes are needed to tamponade the detached retina after vitrectomy when treating retinal detachments. However, several drawbacks associated with current vitreous substitutes have been reported. In the present study, we developed a colorless, transparent and injectable hydrogel as a vitreous substitute that was formed by oxidated HA (oxi-HA) and adipic acid dihydrazide (ADH). HA, one of the essential extracellular matrices of the vitreous body, was oxidized with sodium periodate to create aldehyde functional groups and the derivatives that were characterized by FTIR analysis. The oxi-HA aldehyde group could be crosslinked with ADH hydrazide to form oxi-HA/ADH hydrogel. The rheological results showed that the hydrogel could remain in the solution at 4℃ over 8 min, and then allow rapid gel formation within 3 min upon heating to 37℃; therefore, it is an injectable material. Moreover, the refractive index of this hydrogel ranged between 1.3420 and 1.3442, which is quite similar to human vitreous. The results of biodegradation demonstrated that the hydrogel could maintain its gel matrix over at least 35 days depending on the ADH concentration. In addition, the biocompatibility was evaluated on a retina pigmented epithelium (RPE) cell culture following ISO 10993-5 (tests for in vitro cytotoxicity), and the hydrogel was found to be nontoxic. This study suggested that the injectable oxi-HA/ADH hydrogel could fulfill many critical elements that are desirable in vitreous substitutes.

Session No.: S06-02 Keynote Speaker

In Vitro 3D Tissue Construction

Shoji Takeuchi[1]

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3D tissue construct is important not only in regenerative medicine but also drug testing without animal experiments. Here, I will discuss several MEMS/Microfluidic-based approaches for the rapid construction of 3D tissue. We demonstrated a bottom-up tissue construction method using different types of cellular modules that serve as building blocks for thick and dense 3D tissues (eg. cell beads and cell fibers).

Session No.: S06-03 Invited Speaker

In-situ Transdermal Hydrogel Formation and Cell Delivery Using Near Infrared Light

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[1]Gwangju Institute of Science and Technology

Light-induced polymerization has been widely used to form a hydrogel and to encapsulate cells and support their growth in three-dimensional environments. However, common light sources (i.e., ultraviolet and visible light) strongly interact with biological systems and are therefore inappropriate for in vivo applications, such as transdermal polymerization. In this study, using near infrared (NIR) light that minimally interacts with living tissues, we investigate NIR light-assisted photothermal polymerization (NAPP) of diacrylated polyethylene glycol (PEGDA), in which interactions between NIR light and gold nanorods (GNRs) activate a thermal initiator (i.e., AIPH), resulting in generation of radicals for polymerization of PEGDA. Gelation conditions are developed to minimize the use of initiator and temperature increases (< 43℃) during NAPP. Cell viability is as high as 80% after NAPP-based encapsulation. Incorporation of PEG modified with a cell-adhesive peptide moiety (Arg-Gly-Asp) into the gel system further enables prolongation of cell viability during incubation up to 7 days. NAPP results in successful transdermal gelation and good viability of the transplanted cells. Furthermore, we introduce efficient and controllable thiol-acrylate reactions to induce gelation via a mixed-mode reaction, which could further lower NIR laser power and enhance gelation efficiency to achiever more biocompatible reaction conditions. Thus, this new remote light-triggered in situ gelation and cell encapsulation approach, demonstrated for the first time in this study, will benefit various applications, including cell delivery and remote control over cellular environments.

Session No.: S06-04

Composite Hydrogel Micro-environments with Tunable Mechanical Properties for Single Cell Source Multi-structural Tissue Engineering

Jeroen Rouwkema[1], Ilyas Inci[2], Ajaykumar Vishwakarma[2], Ali Khademhosseini[2]

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It is well known that the differentiation of mesenchymal stromal cells (MSCs) can be controlled by adapting the mechanical properties of the matrix in which the cells reside. This enables the engineering of multi-structural tissues by controlling local mechanical properties. For this purpose, we have developed a novel photocrosslinkable hydrogel platform consisting of gelatin methacryloyl (GelMA) and poly(ethylene glycol) dimethacrylate (PEGDMA). In this study, the concentration of GelMA remained constant at 5% w/v in order to have a constant availability of bioactive sites for cell attachment. The concentration PEGDMA was varied between 0% and 20% w/v, resulting in hydrogels with a compressive modulus ranging between 0.46 ± 0.15 kPa and 291 ± 32 kPa. Human MSCs were incorporated in the gels and cultured in basic medium without differentiation factors for up to 7 days. Apart from that, micro-environments consisting of different hydrogel compositions containing MSC were prepared and combined using photomasks to demonstrate the concept of multi-structural tissue engineering. MSCs incorporated in the gels showed good viability. Organization, as well as differentiation of the cells, depended on the mechanical properties of the gels. Soft gels resulted in elaborate organization of the MSCs and upregulation of the neural marker β3-tubulin, while MSCs remained rounded and expressed the osteogenic marker ALP in stiffer gels. This hydrogel platform offers a photopolymerizable, versatile, and tunable system to control the differentiation of human MSCs in vitro. By combining shaped hydrogel environments of different compositions, local differences in cell differentiation patterns can be induced. The combination of this approach with additional differentiation cues such as growth factor localization can enable the engineering of multi-structural tissues using a single cell source. ACKNOWLEDGEMENTS: This research has received funding from the People Programme (Marie Curie Actions) of the European Union's Seventh Framework Programme under REA grant agreement n°622294.

Session No.: S06-05

Injectable Hyaluronic Acid Based Hydrogel for Nucleus Pulposus Regeneration: An Experimental Study Using an Animal Model

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Low back pain (LBP) is one of the most prevalent musculoskeletal conditions. Four out of five people will experience LBP during their lives. It also causes huge economic burden in society. In the United States, LBP costs about $84.1 billion in direct and $624.8 billion in indirect costs. Intervertebral disc (IVD) degeneration is believed to strongly associate with LBP. Therefore, how to repair the degenerated IVD is considered as an important issue. Early stage of the degeneration originates from the center part of the IVD, called nucleus pulposus (NP). Hence, in this study, we try to develop the oxi-HAG-ADH injectable hydrogel for NP regeneration by using hyaluronic acid and gelatin. Results showed the hydrogel is biocompatible, non-toxic, and easy to use. Besides, it can assist NP cell keep its round shape, and promote various important matrix protein synthesis such as COLII and AGG in vitro. To assess if the hydrogels can regenerated NP in vivo, we used needle-stab model on rat tail to evaluate the treatment outcome. MRI scans, HE stain, alcian blue stain and immunohistochemistry stain were evaluated in the study. Compared with degenerated IVD, the signal intensity of T2-weighted image of the hydrogel injection IVD is much higher at 6 weeks after treatment, and the immunostaining intensities of AGG, SOX-9, S100 and COLII were also increased. The matrix of the hydrogel injection IVD can be stained in dark blue color represents the formation of proteoglycan. The cell morphology of the hydrogel injection disc was similar to control. In conclusion, the hydrogel can assist NP tissue regeneration at the early stage of IVD degeneration. IVD degeneration cascade can be slow down due to the contribution of these cell contained hydrogels. The oxi-HAG-ADH hydrogel could be a promising cell carrier for NP cells in the treatment of early stage IVD degeneration.

Session No.: S06-06

Oct-based Improvement of Geometrical Controllability of 3D-bioprinted Hydrogel Porous Scaffolds

Ling Wang[1], Li Luo[1], Meng-Jie Huang[1], Qing-Qing Zhou[1], Ming-En Xu[1]

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The combined potential of hydrogels and three-dimensional (3D) bio-printing technologies has been an exciting route in developing tissue engineering scaffolds. For tissue engineering scaffolds fabricated by 3D bio-printing, the internal porous geometry is tailed to obtain desired geometrical, mechanical or fluid transport properties. However, because of the inherent characteristics of the hydrogels, it is inherently difficult to produce customized porous structures matching closely the envisioned morphological and physical requirements. The aim of this study is to optimize the robustness and controllability of the 3D printed hydrogel scaffolds by iteratively reducing the mismatch between designed and as-printed. For this purpose, a feedback loop approach based on optical coherence tomography (OCT) in vivo online quantitative evaluation was performed two times. The first run, the hydrogel scaffolds with different pore sizes were designed, printed, imaged and analyzed by custom built swept source OCT system. The quantitative characterization was based on the following morphological parameters: pore size, strut thickness, porosity, surface area and scaffold volume, and compared with the original design parameters. The second run, the mismatch between the designed and as-printed morphology was used as input, and difference correlation analysis and 3D printing process optimization were also integrated to enable a decrease of the mismatch. The effectiveness of the compensation was verified at the end of the printing run with optimized printing parameters setting. For example, for the averaged pore size, the mismatch decreased from 51% to 6%. It concludes that, OCT can further expand its application in the field of tissue engineering, and may be a key tool for scaffold design and characterization, 3D bio-printing process control, in vitro monitoring the development of engineered tissues.

Session No.: S07-01 Keynote Speaker

Evaluation of Properties of Modified Acrylic Bone Cement for Vertebroplasty

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Vertebroplasty has been widely accepted as a therapeutic strategy for painful osteoporotic compression fractures. In this procedure, bone cement is percutaneously injected under pressure into a vertebra through a cannula. Poly(methyl methacrylate), PMMA, has advantages such as low viscosity, easy to perfuse, sufficient ability to strengthen and stiffen vertebral body quickly. With relatively cheaper price, PMMA is the most commonly used filling material in vertebral augmentation procedures. There are some kinds of commercial PMMA bone cement including Simplex-P, HR-V, Palacos R, DePuy 1 (CMW), and Osteobond. Basically, the chemical composition of PMMA bone cement consists of a solid powder and a liquid component. All bone cements on the market fulfill the basic requirements for an orthopedic implant, but there are differences in their properties because of the different powder and liquid compositions. Mechanical stabilization of fractured vertebra and thermal necrosis of nerve endings are the main reasons of postoperative pain relief. Many researchers have attempted to solve these problems by incorporating additional agents into conventional ingredient of the acrylic bone cement. The chemical and physical properties of modified acrylic bone cement of our group are presented in this speech.

Session No.: S07-03

High Purity Dicalcium Phosphate Powder for Dental and Bone Regeneration Made from Oyster Shells at Room Temperature

Sang-Mo Shin[1], Jeong Ho Park[1], Yeon Seoung Lee[1], Dong Min Kim[1]

[1]National Hanbat University

Calcium phosphate compounds exist in various forms. Among them, dicalcium phosphates exist in three different crystalline forms: anhydrous monetite, dihydrate brushite, and hemihydrate. Dicalcium phosphates are used in various applications. They are used as additives in breakfast cereals, flour, and toothpaste. They are also used as dental cements and dietary calcium supplements. They can be converted to hydroxyapatite to make alloplastic grafts for dental applications and bone grafting. For medicinal applications, the purity of calcium phosphate is important due to potential infections or immune rejections from impurities. Traditionally, dicalcium phosphates were prepared from calcium hydroxide which involves high temperature calcination or calcium chloride. In this presentation, we would like to introduce an environmentally friendly low-cost process to prepare very pure dicalcium phosphate nano-powder from waste oyster shells at room temperature.

Session No.: S07-04

Diatoms as Silicon Donors: Application in Bone Tissue Engineering

Antonella Motta[1], Duy Hanh Thi Le[1], Volha Liaudanskaya[1], Walter Bonani[2], Claudio Migliaresi[1]

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Diatomite is one of the most abundant natural sources of hydrated amorphous silica resulting from the accumulation of diatom skeletons. Diatoms possess particular features in structure, morphology as well as composition. Interestingly, it has been recognized that the formation process of diatom skeleton is possibly related to that of human bone. In this study, we have proposed diatoms as silicon donor additives in scaffolds for bone tissue engineering, having been demonstrated the important role of silicon in bone formation. Diatom microparticles (DMPs) and nanoparticles (DNPs) were successfully produced by fragmentation of purified diatoms under alkaline condition. Our result showed that both DMPs and DNPs were able to release silicon, as detected in-vitro by inductively coupled plasma optical emission spectrometry (ICP/OES). In addition, diatom microparticles and nanoparticles - derived from diatom skeletons - showed minimal or non-cytotoxic effects in-vitro as determined by lactate dehydrogenase assays. A series of fibroin scaffolds loaded with different amounts and size of diatom particles (microparticles, nanoparticles and their combination) were fabricated by using the salt leaching method. Diatom particles addition was seen to influence scaffold morphology and mechanical properties, and its biological behavior as assessed on human osteosarcoma cell line MG63 cultures. Scaffolds loaded with diatom particles strongly enhanced cell adhesion, metabolic activity and proliferation. Moreover, the possible beneficial effect of the addition of diatoms particles to silk fibroin on early bone formation was determined through collagen type I synthesis evaluation, osterix expression and alkaline phosphatase induction. Cultures with human mesenchymal stem cells demonstrated the silk/diatom particles scaffolds were able to induce the differentiation of progenitor cells. In conclusion, our findings provided strong evidence for a potential use of diatom-derived particles for biological applications, in particular for bone tissue regeneration.

Session No.: S07-05

Preparation and Characterization of a Family of Resorbable Fracture Fixation Devices

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Metallic internal fixation systems are widely used to fix bone fractures requiring the operative treatment, because they can provide a mechanical support at the initial stage of fracture healing. Nontheless, the devices based on metal alloys often need a second surgery to remove implants, which in some cases can be complicated by integration with bone, cold welding, and screw head destruction. Metallic devices may also cause the decline of bone density resulted from stress shielding. The application of biodegradable plates and screws for fracture fixation has circumvented above problems to improve bone remodelling. Currently poly (L-lactic acid) (PLLA), polyglycolic acid (PGA), poly (lactic-co-glycolic acid) (PLGA), and composites between calcium phosphate ceramics and these polymers are the mostly used materials for resorbable fixation devices. In this study, we report to use a novel polyster for fabricating a resorbable fracture fixation. Our findings showed that screws and plates exhibited a sufficient mechanical strength and controlled degradation property. All these properties can be turned by controlling some processing parameters, such as reaction temperature and time. Cell compatibility was confirmed by successfully supporting attachment and proliferation of rat bone mesenchymal stem cell. Furthermore, the shape of plates can be readily adjusted to fit the repair site after treated under eighty centigrade degree. Consequently, these resorbable devices show a great potential for osseous fixation due to their excellent biodegradation, suitable mechanical property as well as easy shape molding.

Session No.: S07-06

An Alternative Approach for Alveolar Bone Regeneration: Bone Marrow Aspirate Concentrate Combined with Beta-tricalcium Phosphate Granules

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Alveolar cleft is the most common congenital bone defect. Autologous iliac crest bone graft (ICBG) is the most widely adopted procedure, but it associates with door-site morbidities. This study, for the first time, used bone marrow aspirate concentrate (BMAC) combined with Beta-Tricalcium Phosphate (β-TCP) granules to repair alveolar bone defect. The effectiveness of this technique has been compared with autologous ICBG in 12 month follow-up. The bone formation volume was quantitatively evaluated by three-dimentional computed tomography and computer aided engineering technology. The results showed that BMAC/β-TCP granules grafting was radiographically equivalent to ICBG in alveolar cleft repairing. Although considerable resorption was observed in BMAC/β-TCP grafting until 6 months after surgery, no significant difference was found in Chelsea score and bone formation volume between groups. These finding indicate that BMAC/β-TCP grafting is a safe and effective approach for alveolar bone regeneration.

Session No.: S08-01 Keynote Speaker

From Tissue Engineering to Regenerative Medicine. Learning from Natural Tissue Healing: Identification of a New Cell Population Constitutively Circulating in Healthy Conditions and Endowed with a Homing Ability Toward Injured Sites

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With few exceptions, for the repair of organ and tissue defects and damages, cell therapy and tissue engineering approaches never really took-off. Main bottlenecks are: i) logistic of collecting from patients, expanding in culture and returning the cells to the surgical theater; ii) high cost of the culture procedure within the GMP facilities required by the strict rules defined by National and European Regulatory Agencies. Therefore, it appears that a “classical” tissue engineering approach should be considered only in extreme critical situations and that new therapeutic strategies should be developed in order that a large number of patients could benefit of them. The rapidly increasing knowledge about the body physiological response to injury suggest that the human organism itself could provide the crucial elements needed for tissue repair and regeneration. To stimulate the intrinsic endogenous potential of a tissue to heal or regenerate, we propose, an “off the shelf” product, obtained by the integration of a biomaterial scaffold with a Platelet Lysate (PL), a source of growth factors in the “right” composition and in the “right” concentrations, as well as stem cell conditioned culture medium or released microvesicles. We also report on the identification of a rare population of cells present in the peripheral blood that actively participates in the tissue repair/regeneration process. Injury signals are sufficient to (i) specifically direct recruitment to the wound site of these Circulating Healing (CH) cells; (ii) promote their differentiation and appropriate integration in the regenerative microenvironment. CH cells were identified by an innovative flow cytometry strategy as small cells not expressing CD45 and lineage markers. The analysis of their global transcriptome revealed their uniqueness when compared to other cells characterized by varying stemness degree. Moreover, CH cells presented a high expression of key pluripotency-associated genes and markers of the epiblast developmental stage.

Session No.: S08-02 Invited Speaker

Regulation of Basement Membrane Dynamics in Engineering Tissue Structure Formation of Glandular Organs

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Structure formation is tissue-specific and important for physiological function. Many organs responsible for secretion, nutrition supply, or metabolite exchange, are featured by ramified tissue architecture. The branching structure efficiently enlarges the surface for biological reaction that benefits multicellular organism for metabolite and nutrient exchange. The salivary gland is a branching organ important for saliva secretion and regulation. A considerable body of research indicates that salivary gland branching results from epithelial-mesenchymal interaction, and accordingly depends on support of basement membrane (BM). We had previously demonstrated the efficacy of chitosan-based biomaterials in engineering branching structure formation of the salivary glands. Further attempt was explored to regulate BM dynamics to facilitate structure formation of engineered glandular organs. It was found that chitosan effect diminished when BM components were removed from cultured salivary gland. With chitosan, BM components and corresponding receptors increased in amounts, and expressed in tissue-specific manners beneficial for branching formation. The chitosan mediated effect decreased when either BM components or receptors were removed. Chitosan effect also disappeared when downstream signaling of BM components and receptors were inhibited. Taken together, the data revealed the branching promoting effect of chitosan on developing salivary glands is dependent on BM dynamics. By regulating BM components and receptors, chitosan efficiently stimulates downstream signaling to facilitate salivary gland branching. This study revealed the underlying mechanism accounting for chitosan effect in engineering of salivary gland structure, which pave ways for further optimization and application of chitosan-based biomaterials in tissue engineering of the glandular organs.

Session No.: S08-05 Invited Speaker

Hydrogel Mechanics-mediated 3D Organoid Assembly

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A series of organoids, small-sized three-dimensional buds with similar microstructure and functionality of organs of interests, have emerged as promising platforms to regulate cellular emergent behavior and to evaluate newly developed drug molecules and biomedical tools. These organoids are being also explored to assemble biological machine with self-recognition and actuation capabilities. Stem cell clusters, such as embryoid bodies (EBs) derived from embryonic stem cells, are being extensively studied to create functional organoids because of the pluripotency of cells. It is common to control phenotypes of ES cells with varying soluble molecular compounds; however, there is still a need to improve the controllability of cell organization and differentiation, and thus, the quality of created organoids. This talk will present a simple and unprecedented strategy to promote formation of contracting cardiovascular organoids and three-dimensionally inter-linked neurospheres by modulating the stiffness of a cell adherent hydrogel. Using collagen-conjugated hydrogels with controlled elastic moduli, we discovered that co-differentiation of ESCs in the cell cluster was maximal on the gel with the stiffness similar to soft tissue. We are further using the resulting neurospheres to assemble neuromuscular junction in vitro. We envisage that the results of this study will greatly contribute to expediting use of stem cells for both fundamental and applied bioscience studies and also to creating novel biomedical platforms.

Session No.: S08-06 Invited Speaker

Paper-based Bioactive Scaffolds for Stem Cell-mediated Bone Tissue Engineering

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Bioactive, functional scaffolds are required to improve the regenerative potential of stem cells for tissue reconstruction and functional recovery of damaged tissues. Here, we report a paper-based bioactive scaffold platform for stem cell culture and transplantation for bone reconstruction. The paper scaffolds are surface-engineered by an initiated chemical vapor deposition process for serial coating of a water-repellent and cell-adhesive polymer film, which ensures the long-term stability in cell culture medium and induces efficient cell attachment. The prepared paper scaffolds are compatible with general stem cell culture and manipulation techniques. An optimal paper type is found to provide structural, physical, and mechanical cues to enhance the osteogenic differentiation of human adipose-derived stem cells (hADSCs). A bioactive paper scaffold significantly enhances in vivo bone regeneration of hADSCs in a critical-sized calvarial bone defect. Stacking the paper scaffolds with osteogenically differentiated hADSCs and human endothelial cells resulted in vascularized bone formation in vivo. Our study suggests that paper possesses great potential as a bioactive, functional, and cost-effective scaffold platform for stem cell-mediated bone tissue engineering. To the best of our knowledge, this is the first study reporting the feasibility of a paper material for stem cell application to repair tissue defects.

Session No.: S08-03

Fabrication of Oral Mucosal Epithelial Cell Sheets Using Explant Culture

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Objective: Human oral mucosal epithelial cell (hOMEC) sheets have been successfully used to treat epithelial defects of the cornea and esophagus. In conventional research, cell source of the epithelial cell sheets have been collected by using enzyme. The cells are then seeded on cell culture inserts, as a primary culture. However, the size of resectable tissue is limited, restricting the resultant cell numbers. Therefore, it is important to establish a subculture method for generating sufficient numbers of cells for hOMEC sheet fabrication. The aim of this study was to fabricate and examine a hOMEC sheet cultured on a temperature-responsive surface via an explant culture method. Method: Human excess oral mucosa was harvested by tonsillectomy and then minced as finely as possible. The tissue was placed in a culture dish coated with type I collagen and subjected to primary explant culture in keratinocyte growth medium (KCM). Subsequently, the cultured cells were trypsinized and seeded onto a temperature-responsive cell-culture insert at a density of 8–60×10^4 cells/cm^2. In addition, we examined whether hOMEC sheets could be generated from frozen cells. The sheets were analyzed by histology and flow cytometry. Results: We observed approximately 80% increase in cell proliferation between days 12 and 16 of primary cell culture. The efficacy of our approach was comparable with conventional methods, yielding five times as many or more cell sheets than these methods. The higher the number of harvested cells used for seeding, the shorter the time required for cell sheet generation. It was also possible to generate cell sheets using cells that had been frozen for 2 months. Conclusion: We have demonstrated that tissue-engineered epithelial cell sheet grafts can be fabricated using temperature-responsive culture via the explant culture method. We believe that the new method would be useful to create cell sheets for clinical application.

Session No.: S08-04

Segmental Trachea Scaffold Tissue Engineering: From Full to Partial Decellularization

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The trachea is critical for respiration and airway protection in humans. Ideal methods for the reconstruction of the tracheal structure and restoration of tracheal function have not yet been developed once the trachea has been damaged or removed. In our previous attempts we evaluate the feasibility of using a whole segment decellularized tracheal scaffold to reconstruct the trachea. Under the rabbit model, trachea scaffolds were created using our previously developed freeze-dry-sonication-SDS (FDSS) decellularization process and transplanted orthotropically into segmental tracheal defects. We found that the FDSS decellularization process is effective in creating whole-segment, sub-totally decellularized trachea scaffolds. However, although the respiratory epithelium regeneration on the inner surface appeared to be satisfactory, the tubular structures were not able to be maintained after transplantation, which ultimately led to the death of the animals. Therefore, it seems that the cartilage component appear to be very critical and it seems that host cell migrations and angiogenesis are difficult to happen once a complete decellularized tracheal cartilage scaffold is transplanted. Therefore a new protocol was developed with a quick, partial decellularization of the harvested trachea. The idea is based on the low immunogenicity of the cartilage tissue and it is possible to implant the scaffold without serious rejection responses even with some donor cartilage cells remaining alive. By leaving at least a portion of live cartilage cells in the transplant, it is possible that these live cells continue to function and contribute to a higher structural strength after transplantation which is critical for maintaining the tracheal tubular structure. Preliminary data showed cartilage cells remain alive under vital stain through our new decellularization protocol. One month after implantation the implanted tracheal scaffold remained intact, especially the cartilage portion, without signs of rejection.

Session No.: S09-01 Keynote Speaker

Biomaterials Strategies to Enhance the Therapeutic Potential of Human Neural Stem Cells for Brain Tissue Regeneration

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Transplantation of neural stem or progenitor cells (NSCs) represents a promising strategy to reconstruct the lesion cavity and promote brain tissue regeneration following traumatic brain injury. However, ongoing inflammation at the lesion site and the lack of supportive matrix structure and vasculature within the cavity present a hostile environment that results in low cell survival and poor control over differentiation and engraftment of the transplanted cells. We optimized an in situ crosslinkable hyaluronic acid (HA) hydrogel conjugated with laminin-derived peptide as a cell delivery matrix. It generated a robust neovascular network within the hydrogel at the lesion cavity when injected at 3 days following the CCI injury in a rat TBI model, and promoted significant vasculature network formation filling the lesion site at 4 weeks to 6 months following hydrogel implantation. Using this tailored HA hydrogel, delivering hNSCs in the format of spheroids for brain regeneration was superior to single cell transplantation in promoting cell survival following implantation. The majority of the grafted cells differentiated to Tuj1+ and MAP2+ neuronal progenitors and populated the entire lesion cavity. The human NSC-derived neuronal progenitors extended large numbers of axons into the host brain tissue. Since manipulation of cell transcriptional network is a more effective approach to promote stem cell differentiation, when compared to growth factors cocktails, we optimized a poly (β-amino ester) (PBAE)-based nanoparticle system to transfect hNSCs with a key transcription factors Neurogenin-2 (Ngn2) and generated a significantly larger number of neurofilament positive (NF+) mature neurons at the lesion site of CCI at 4 weeks post transplantation when compared to non-transfected cells. This approach holds great potential in promoting neuronal maturation in a tissue repair site, thus improving therapeutic outcomes of stem cell-based therapy for brain tissue regeneration.

Session No.: S09-03

Restoration of Urinary Sphincter Using Adipose Stem Cells Plus Platelet-rich Plasma in a Pudendal Nerve-transected Rat Model of Stress Urinary Incontinence

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INTRODUCTION AND OBJECTIVES: Adipose derived stem cells (ADSC) are nonimmunogenic and have the ability to self-renew and to differentiate into multiple cell types. They has been used to restore urinary sphincter function in animal models. But there is not strong evidence regarding the role of the vehicle that must be used in combination with ADSC to improve effectiveness at the injection site. Objective: To assess whether adipose stem cell (ADSC) plus platelet rich plasma (PRP) could promote urethral sphincter restoration in a stress urinary incontinence (SUI) rat model. METHODS: Thirty five female inbred Wistar rats were used in our study. Animals were divided into seven groups (five animals per group): continent (C), sham (S), PNT (D), PNT+PBS injection (P), PNT+PBS+ADSC injection (PA), PNT+PRP injection (R) and PNT+PRP+ADSC injection (RA). Twenty five females rats underwent bilateral pudendal nerve section (PNT) to induce SUI. ADSCs were purified from fat tissue of a 4-week-old inbred male Wistar rat, labeled CM-Dil and injected into the urinary sphincter in twelve oÂ´clock position with 70 microliltres of PBS or PRP. Four weeks after injection, cystometry was undertaken in all animals and leak point pressure (LPP) measured to assess urethral resistance function. All groups were sacrificed after cystometry, urethra sections were submitted for histology, immunohistochemistry assessment. RESULTS: LPP was increased significantly in R, RA and PA animals after implantation ($P < 0.01$), but was not different from group C and S. Histological and immunohistochemical examination demonstrated increased numbers of surviving ADSCs increased muscle/collagen ratio as well as increased microvessel density at the injection sites in RA compared to PA animals (CM-Dil +). CONCLUSIONS: PRP may potentially improve the action of transplanted ADSC to restore the histology and function of the urethral sphincter in a SUI rat model

Session No.: S09-04

Using Adipose-derived Stem Cell Toward Osteocyte Differentiation with Small Intestine Submucosa (SIS) for Auricular Reconstruction

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Ear reconstruction remained a challenge for the plastic surgeons. Using autologous cartilage graft has problems of donor site availability and morbidity. The tissue engineering technique provides another route in ear reconstruction Current tissue engineering methods in auricular reconstruction focused on synthetic scaffolds combined chondrocytes. But there are problems existed such as the cells manipulated in vitro lose their cartilage-specific phenotype, lack of blood and nutrient supply results in cell death and subsequent loss of shape and function, the degradation products of synthetic scaffolds could cause inflammatory reaction and negatively affect neocartilage formation in vivo. Furthermore, no ideal tissue engineering constructs now can withstand the contractile forces exerted by skin and surrounding tissue during normal wound healing. In the present study, we designed a tissue engineering auricular constructs by culturing human adipose stem cell toward osteocyte differentiation on the porcine decellularized small intestine submucosa in vitro. We evaluated the cell growth potential and mechanical properties. Then an ear-shaped constructs were created by rolling, tucking and suturing of cell-seeded SIS in vitro and then implanted in the nude mice back. The histology, inflammation reaction, neovascularization, mechanical properties and maintenance of ear shape were investigated. The results showed that although not using chondrocyte, the combination of human adipose stem cell with small intestine submucosa could provide a vascularized ear-shaped constructs in vivo. The mechanical property and shape were maintained for 12 months. This method could be an alternative method of tissue engineering auricular reconstruction.

Session No.: S09-05

Accurate Shape-designed Scaffolds for Human Auricular Tissue Engineering

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Engineered cartilage is a promising option for auricular reconstruction.Due to the lack of appropriate scaffolds, auricular reconstruction remains one of the greatest challenges in plastic surgery. The major technical bottlenecks that impede clinical translation of this approach is how to retain the accurate ear shape in skin-pressure environment after implantation. Although our group preliminary study confirmed the mechanical strength of PGA scaffold was enhanced by coating with polylactic acid (PLA), how to overcome its limitations in achieving desirable mechanical strength and accurate control over shape remains an unsolved problem. In this study, we improved the previous ear-shaped model. In experimental group，the ear scaffold used PCL mash as inner core, which was wrapped with PGA fibers, and coated with PLA. And control group used the same weight PGA and PLA.Both group was seeded with microtia chondrocytes.Cell-material compound was tested by gross view, laser scanning,histology,immunohistology after been cultured in vivo and in vitro.The results shows that , the constructs in experimental group largely retained the original shape during cultured in vitro for 12 weeks ,and formed ear-shaped cartilage-like tissues, which revealed a tissue structure with abundant cartilage extracellular matrices and mature lacuna. Additionally, the ear-shaped cartilage implanted in a nude mouse model for 12 weeks maintained not only in shape but also in size and flexibility. However,the control group`s compound was wraping , and lost its original shape. These results may provide a useful strategy for reconstructing cartilage tissue with complicated shapes such as external ear reconstruction.

Session No.: S09-06

Bio-mimetic Waterproof Protein Glue for Urinary Fistula Treatment

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Urinary fistulas, abnormal openings of urinary tract organ, are serious complications that, if unresolved, worsen the quality of life. Particularly, in many underdeveloped countries where urinary fistulas devastate millions of young lives, conventional management strategies are not satisfactory. For more effective and non-invasive fistula repair, fluid-type tissue adhesives or sealants have been suggested. However, conventional products do not provide a suitable solution due to safety problems and/or poor underwater adhesion under physiological conditions. Herein, we proposed a unique water-immiscible mussel protein-based bioadhesive (WIMBA) exhibiting strong underwater adhesion which was employed by two adhesion strategies of marine organisms; 3,4-dihydroxy-L-phenylalanine (DOPA)-mediated strong adhesion of mussel and water-immiscible coacervation of sandcastle worm. Good performance of the developed biocompatible WIMBA was confirmed in both ex vivo and in vivo fistula model. The WIMBA with good durability and high compliance successfully sealed and repaired fistulas in animal model as compared with positive control group (suture). Collectively, WIMBA could be used as a promising sealant for urinary fistula with further expansion to diverse internal body applications.

Session No.: S10-01 Keynote Speaker

Sticky Mussel Proteins as Innovative Regenerative Medical Biomaterials

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Marine mussel adhesion is known to be mediated by sticky proteins, which are secreted through the mussel byssus and have great potential as biologically and environmentally friendly adhesive biomaterials due to their biocompatibility and biodegradability. In addition, mussel glues have strong adhesion ability even on wet surfaces due to unique amino acid arrangements and composition. However, researches using the natural amino acid composition have been limited due to difficulties in obtaining sufficient quantities of mussel glues for practical applications. Previously, we successfully produced genetically redesigned new fusion glue protein using a bacterial expression system, and this fusion glue showed significant adhesion ability. In this talk, we present a unique water-immiscible mussel protein-based bioadhesive exhibiting strong underwater adhesion which was employed by two adhesion strategies of mussels; Dopa-mediated strong adhesion and water-immiscible coacervation. The developed biocompatible underwater bioadhesive successfully sealed ex vivo urinary fistulas and provided good durability and high compliance. Thus, the developed bioadhesive could be used as a promising sealant for urinary fistula management with further expansion to diverse internal body applications. In addition, we present a photo-activated recombinant MAP-based hydrogel bioadhesive inspired by insect dityrosine crosslinking chemistry. The developed hydrogel bioadhesive exhibited substantially stronger bulk wet tissue adhesion than commercially available fibrin glue and good biocompatibility in both in vitro and in vivo studies. Besides, the easily tunable, blue light-activated crosslinking enabled an effective on-demand wound closure and facilitated wound regeneration. Based on these outstanding properties, the MAP-based photo-curable hydrogel bioadhesive holds great potential as an ideal medical glue for diverse medical applications, including sutureless wound closures of skin and internal organs and effective regeneration of wounded tissues.

Session No.: S10-02 Keynote Speaker

Bioactive Materials for Tissue Engineering

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It is known that the chemical composition and nano-structure are two critical factors of biomaterials which could affect cell behavior and tissue regeneration. We have designed and fabricated calcium phosphate and silicate based bioactive ceramics and composites with different chemical composition and micro structures, and demonstrated that the chemical signal released from the silicate based biomaterials and microstructural environment of calcium phosphate bioceramics stimulated stem cell behavior such as proliferation and differentiation indicating that “tissue inducing biomaterials” with certain chemical composition and surface structure may be designed for tissue engineering applications.

Session No.: S10-03 Keynote Speaker

Marine Inspired Biomaterials as Building Blocks for Tissue Regeneration

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Nature has shown superb examples of functional biomaterials and bioactive compounds, being explored nowadays in the biomedical arena. Marine derived antitumoral compounds and mussel-inspired adhesives are key examples from the marine environment, but tissue engineering is building its share. In fact, not only several marine origin materials are being used as building blocks for the manufacture of tissue engineering scaffolds [1], as well as marine organisms' astonishing architectures are serving as inspiration for cell culture towards tissue recovery and regeneration [2, 3]. Examples of different marine origin polymers will be discussed, exploring different processing technologies towards the proposal of scaffolds and membranes for tissue and organ therapy. Focus will be given to: (i) squid chitosan [4], a more reactive biopolymer making easier to control deacetylation degree and thus cell adhesion; (ii) to macroalgae sulfated polysaccharide fucoidan, a bioactive biopolymer showing potential as drug to tackle breast cancer but also as structural component of photocrosslinked particles for sustained drug release or cell encapsulation in diabetes therapeutic approaches; and (iii) marine origin collagen [5], from fish skins (codfish, Atlantic salmon and blue shark), used on the development of membranes, sponge-like structures and composites to tackle wounds or cartilage and bone damage pathologies. Finally, key examples of marine phenomena – as the dynamic collagenous tissues – are being studied as inspiration for the development of functional and smart biomaterials. References: [1] Int Mater Rev 57 (2012) 276; [2] Tissue Eng 9 (2003) 1159; [3] Cryst Growth Des 14 (2014) 4545; [4] Biomed Mater 8 (2013) 045002; [5] Marine Drugs 12 (2014) 5881. Acknowledgements: Funds from ERDF under project 0687_NOVOMAR_1_P (POCTEP – INTERREG 2007-2013) and by European Union through European Research Council – Project ComplexiTE (ERC-2012-ADG 20120216-321266) and Framework Programme for Research and Innovation Horizon 2020 – Project SponGES (H2020-BG-01-2015-679849).

Session No.: S10-04

Designing Carbohydrate Biomaterials to Modulate Macrophage Behaviour for Immunotherapy and Tissue Regeneration

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Macrophages play diverse and essential roles in a variety of physiological and pathological processes, notably including bone development and cancer progression [1]. Designing biomaterials to modulate the behaviour of macrophages - and harness their functions - may provide new approaches to solve various unmet medical challenges [2-4]. Here, we demonstrate our development of a series of carbohydrate polymers for targeting, eliminating, and/or modulating the phenotype of, macrophages. Most of them have glucose and mannose units in their backbones – hence named Glumax, which endow them with affinities for macrophage carbohydrate receptors, including mannose receptor (CD206 in human), toll-like receptor 4 (TLR4) or dectin-1. The Glumax polymers are developed into various forms such as nanoconjugates, injectable gels or scaffold coatings; or are chemically modified for direct use as polymer therapeutics. For instance, Glumax-B11 / bisphosphonate conjugate exhibited high efficiency in targeting and selectively eliminating tumour-associated macrophages (TAMs) [5]. Another example is Glumax-B2e, an injectable polymer engineered into either three-dimensional (3D) gel or coating materials for scaffolds. It can recruit and activate monocytes in a controlled manner, stimulating the expression of pro-osteogenic cytokines (e.g. Oncostatin M) and promoting osteogenic differentiation of mesenchymal stem cells. In summary, these polymers demonstrate unique functions in modulating macrophage behaviour that may have immediate impact in various immunotherapeutic and tissue regenerative applications. Acknowledgements: We thank supports from the Macao Science and Technology Development Fund (FDCT 048/2013/A2) and University of Macau (MYRG2014-00069-ICMS-QRCM; MYRG2015-00160-ICMS-QRCM). Reference: [1] L Dong, C Wang *. Trends Biotechnol 2013, 31, 342; [2] Z Huang, C Wang et al. Biomaterials 2015, 48, 26; [3] W He, CM Wang * et al. Sci. Rep. 2016, 6, 24506; [4] C Wang, L Dong *. Trends Biotechnol. 2015, 33, 10; [5] X Zhan, C Wang * et al. Biomaterials. 2014, 35, 10046

Session No.: S10-05

3D Nanofibrous Scaffold and Its Applications

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ECM is highly porous and fibrous in nature and therefore ideally a scaffold for tissue engineering should mimic such environment for optimum interaction with cells. Electrospinning is a versatile method to develop sub-micron fibers but the scaffolds fabricated using this method is usually a highly dense 2D mat which does not allow much cell infiltration, therefore making it unsuitable for scaffold applications. Here we would like to report on our three dimensional (3D) biodegradable, fibrous, ECM mimicking scaffolds. We have applied this scaffold across many applications in soft tissues demonstrating the versatility and potential of such structures. Firstly in the application in chronic wound, it was demonstrated that human dermal fibroblasts (HDFs) easily infiltrated into scaffolds at a depth of ~1400 μm after 7 days culturing, and showed significant progressive proliferation on scaffolds immobilized with collagen type I. In vivo models show chronic wounds treated with scaffolds had a faster closure rate. These results indicate that the 3D bioactive fibrous scaffolds may be a potential wound dressing for chronic wound repair. We also applied these scaffolds for blood capillary engineering. Herein, we developed a novel method to significantly improve vessel density in neovascularized tissue engineered constructs. Endothelial cells (HUVECs) were confluently cultured on resorbable electrospun poly (D, L-lactide-co-glycolide) (PLGA) microfibers of capillary dimensions and then further embedded in collagen with HUVECs and VEGF, then cultured for 30 days before implantation. In vitro results indicated that the fibers provide contact guidance to form primary networks to direct more vessels branching of HUVECs in hybrid constructs and the vessel integrity of microvasculature was retained after fiber degradation. When implanted onto the dorsal skin of immune-deficient mice, these vessels rapidly anastomosed with mice vasculature within a day. These engineered capillaries can be applied to enhance prevascularization in tissue engineered constructs .

Session No.: S11-01 Keynote Speaker

Injection of Human Amniotic Fluid-derived Stem Cells for Urinary Incontinence Therapy

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Treatment for stress urinary incontinence (SUI) has had limited success with frequent adverse reactions. Stem cell therapy has been proposed as an alternative to overcome the limitations and side effects of existing therapeutic procedures.Human amniotic fluid stem cells (hAFSCs) have been reported recently as a promising stem cell source. For the development of hAFSCs as a feasible cell-based medicinal product, pre-clinical studies are mandatory. Major parameters for pre-clinical studies are cell passage number and dose, efficacy, toxicity and tumorigenicity, and cell trafficking in an animal model. This study was performed to assess optimized cell processing and characterization for the first step of the pre-clinical evaluation of human amniotic fluid stem cell (hAFSC) therapy in a urinary incontinence animal model. The proper cell passage number was analyzed with hAFSCs at passages 4, 6, and 8 at week 2. The cell dose optimization included 1×10^4, 1×10^5, and 1×10^6 cells at week 2. The in vivo cell toxicity was performed with 0.25×10^6, 0.5×10^6, and 1×10^6 cells at weeks 2 and 4. Cell tracking was performed with 1×10^6 cells at weeks 2 and 4. Results indicated that the selected optimal cell passage number was smaller than 6, and the optimal cell dose was 1×10^6 for the mouse model. In our pre-clinical study, hAFSC-injected animals showed normal values for several parameters. Moreover, the injected cells were found to be non-toxic and non-tumorigenic. Furthermore, the injected hAFSCs were rarely identified by in vivo cell trafficking in the target organs at week 2. In conclusion, this study demonstrates for the first time the pre-clinical efficacy and safety of hAFSC injection in the urinary incontinence animal model and provides a basis for future clinical applications.

Session No.: S11-02 Keynote Speaker

Using Glycosaminoglycans to Scale-up Mesenchymal Stem Cells for Clinical Use

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Clinical use of mesenchymal stem cells (MSC) for treating conditions where body tissue needs to be replaced, repaired, or regenerated is rapidly becoming a therapeutic reality. Before clinicians can have ready access to this evolving technology, the bottleneck in their ex vivo mass production must be overcome. To generate a typical MSC transplant, these cells are surgically isolated from patients or prospective donors and further processed to achieve the quantities needed for therapeutic use. This bioprocessing step is not only time-intensive but also extremely costly and there is no guarantee that the cells will retain their therapeutic ability after such extensive handling. Strategies that favor the maintenance of naïve MSC after lengthy ex vivo culture-expansion are in high demand. Current practice is to supplement cultures with either growth factor additives or natural/synthetic substrates that can result in an altered cell phenotype and a subsequent loss of clinical efficacy. Our research has focused on developing particular heparan sulfate (HS) glycosaminoglycan sugars that have increased affinity for factors produced by MSCs during their ex vivo culture expansion. When used as supplements that are added into media to culture-expand MSCs, these HS sugars bind and sustain the activity of endogenous factors important in maintaining the purity and potency of MSCs. To date, our efforts have centered on controlling key interactions between endogenous growth factors known to be MSC mitogens, and their cognate cell receptors. Our data shows that MSCs cultured with HS supplementation are highly efficacious when transplanted into rodent osteochondral defects. Thus, by using HS agents to increase particular molecular interactions, our data is showing the power that HS exerts on the cellular microenvironment and highlights the considerable potential this strategy has for bioprocessing of MSCs intended for clinical use.

Session No.: S11-03 Invited Speaker

Transdifferentiation of Human Adipose-derived Stem Cells (hADSCs) into Neuron-like Cells for Cell Replacement Therapy in Middle Cerebral Artery Occlusion Mouse Model

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Human Adipose-Derived Stem Cells (hADSCs) have proved to be a promising stem cell source for treating stroke. hADSC derived neuron like cells (hADSC-NCs) as intermediate cells may work better for cell replacement therapy in stroke rescuing. Previously, minor experimental data demonstrate this point. No agreement on the mechanism of hADSC-NC transplantation for treating stroke in vivo is reached, which hinders their further clinical translational application. To explore the in vivo mechanism, hADSC-NCs were labeled with EGFP expressing FG12 lentivirus and injected into MCAO mouse infarct area by in situ way. Neurological function was evaluated by Rogers Scaling System and their spacial learning and memory was determined by Morris Test. TTC was carried out to compare the infarct area among groups. Histoimmunostaining was used to track the injected hADSC-NCs for their in vivo migration, trans-differentiation and integration with the endogenous neuronal circuitry. Whole brain immune factors were determined to better address the underlying rescuing mechanism. qRT-PCR was performed on neural markers of MBP, MAP2, GFAP, microglia marker of Iba1. It was found that hADSC-NCs could promote both spacial learning and memory of MCAO mice. The introduced hADSC-NCs could survive well and integrate into the endogenous tissue. Co-localization of GFP and MAP2 were found in the whole cortex and the hippocampus. Minor percentage of GFP and GFAP co-localized cells were found. Meanwhile, Iba1+ and GFAP+ cells were significantly ($P<0.05$) suppressed by hADSC injection. IL9 was significantly reduced indicating the immune suppression effects of hADSC-NCs. This study demonstrated that hADSC-NC intervention with MCAO mice could apparently ameliorate stroke symptoms by cell replacement, positive immnunomodulation and promoting the viability of endogenous neurons.

Session No.: S11-04

Adipose-derived Stem Cells Enhance Burn Wound Healing and Neuropathic Pain Treatment

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Stem cells are found in multi-cellular organisms, and are biological undifferentiated cells which can differentiate into specialized cells to divide and produce more stem cells through mitosis. In adult organisms, stem cells and progenitor cells act as a repair system for the body replenish adult tissues. We select to carry on the experiments with adipose-derived stromal/stem cells (ADSCs), because the obtaining on clinic is easy; the chance of surgery complication is low; the patient acceptance is high; the cultured cell number is in a large quantity; and the proliferative generation is stable. Burn injury has been shown to bring out the neuropathic pain in general cases, and it is hard to cure. The present clinical medicine is with low efficacy, and is often accompanied obvious side effects. The management of neuropathic pain after burn injury is a critical issue. We found that the over-inflammation and the neuron apoptosis in spinal cord ventral horn in burn damaged rat. ADSCs can be applied to diminish inflammation, decrease neuropathic pain and reduce neuron apoptosis. Following, we will move to treat the difficult wound (high glucose conditions). During normal wound healing, various kinds of cells are recruited to the wound by cytokines released from the injury area. The prolong inflammation and the poor circulating / resident cell migration impaired the diabetic wound healing. The elevated TNF- expression decreases fibroblast proliferation and increases apoptosis of fibroblast. The local injection around the wound with keratinocyte-secreted cutaneous T-cell attracting chemokine (CTACK) is shown to improve wound healing by recruiting circulating cells. In our clinical preliminary data, the increased TNF- expression with the decreased CTACK expression was noted in diabetic wound fluids compared with the normal wound fluids. This will be a worth topic to discuss the relationships between ADSCs and the wound repair.

Session No.: S11-05

Human Induced Pluripotent Stem Cells Promote Bone-like Structures via Upregulated Expression of Bmps in a Mouse Ectopic Model

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Use of h-iPSCs for tissue engineering and tissue regeneration applications is most appealing, because h-iPSCs are an inexhaustible source of osteocompetent cells. The present study investigated the contribution of undifferentiated h-iPSCs and elucidated aspects of the underlying mechanism(s) of the involvement of these cells to new bone formation. Implantation of undifferentiated h-iPSC seeded on coral particles in ectopic sites of mice resulted in expression of osteocalcin and DMP-1, and in mineral content similar to that of the murine bone. The number of the implanted h-iPSCs decreased with time and disappeared by 30 days post-implantation. In contrast, expression of the murine osteogenic genes at day 15 and 30 post-implantation provided, for the first time, evidence that the implanted h-iPSCs affected the observed outcomes via paracrine mechanisms. Supporting evidence was provided because supernatant conditioned media from h-IPSCs (h-iPSC CM), which had no effect on the migration and proliferation of human mesenchymal stem cells (h-MSCs) in vitro, promoted their osteogenic differentiation. Specifically, h-iPSC CM activated the phosphorylated BMP-SMAD 1/5/8 pathway, induced upregulation of the BMP-2, BMP-4 and BMP-6 genes, and promoted calcium deposition in the cell extracellular matrix. Given the current wide interest in the use of h-iPSCs for regenerative medicine applications, the present study contributes new insights into aspects of the mechanism underlying the bone promoting capability of h-iPSCs; such knowledge can be applied in developing improved methodologies in order to enhance release of BMPs by h-iPSCs in vivo.

Session No.: S11-06

Epigenetic Regulation in Dedifferentiation-mediated Msc Reprogramming: Application in Tissue Repair and Cancer Targeting

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In mammals, the differentiation process was thought to be irreversible. However, recent studies have demonstrated that dedifferentiation may function as an alternative mechanism to achieve tissue regeneration in mammals. Can we induce dedifferentiation readily in culture without gene manipulation and obtain reprogrammed stem cells with improved therapeutic potential? Our previous study demonstrated that MSCs could be reprogrammed in vitro via neural differentiation and dedifferentiation with enhanced therapeutic efficacy in a rat model with ischemic brain damage (Stem Cells, 2011). This is of particular interest, since the finding provides a potential approach to overcome some of the major hurdles faced by current MSC-based therapy. Apart from neural lineage, we have found that after in vitro induction of osteogenic differentiation, MSCs can also be reverted to a primitive stem cell population (dedifferentiated osteogenic MSCs) with enhanced stem cell potency as demonstrated by improved cell survival, colony formation, osteogenic potential and increased expression of pluripotency genes. In addition, we demonstrate that Nanog plays critical role in maintaining the dedifferentiation phenotype, since Nanog-knockdown in MSCs completely reverses the enhanced cell survival and differentiation in De-Os-MSCs. More interestingly, we reveal that the increased expression of Nanog and Oct4 is attributed to the epigenetic activation involving both DNA methylation and histone modifications, as evidenced by decreased methylation and promoter accrual of activating histone marks, such as H3K4me3 and H4ac on gene promoters. Our findings indicate that dedifferentiation can be achieved after different lineage commitment in MSCs (Sci Reports, 2015). Recently, following our previous study, we evaluated the effect of dedifferentiation on the migratory capability of MSCs and their homing ability to glioma. Our results show that dedifferentiation strategy dramatically enhances the migratory and tumor targeting ability of MSCs both in vitro and in vivo. Furthermore, we reveal a novel epigenetic regulatory mechanism involving

Session No.: S11-07

Rap1 Deficiency Impairs Immunosuppressive Potency of Mesenchymal Stem Cells by Decreasing Paracrine Functions

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Background- The immunoregulation of mesenchymal stem cells (MSCs) in the presence of inflammation is starkly divergent to that under normal conditions. The processes that underlie their immunomodulatory activity are complicated and poorly understood. This study aimed to elucidate the role of Rap1 (repressor/activator protein) in regulating the immunomodulatory potency of MSCs in acute allograft rejection of heart transplantation.

Methods and Results- The immunosuppressive potency of wild type (RWTMSCs) or Rap1 deficient (RKOMSCs) MSCs was examined in mice with acute allograft rejection of heart transplantation. Combined with immunosuppressant Rapamycin at a dose of 1mg/kg/d, RWTMSC treatment notably prolonged survival of the transplanted heart compared with RKOMSC treatment ($p<0.01$). Immunosuppressant impairment of RKOMSCs is associated with a decline in the capacity of secreting cytokines and recruiting Treg cells. RKOMSCs displayed a marked insensitivity to inhibit MLR (Mixed Lymphocyte Reaction) in vitro, mainly through impaired cytokine production. Finally, transplantation of encapsulated RWTMSCs, but not RKOMSCs, prolonged survival of the heart allograft.
Conclusions- Rap1 is required for MSCs to maintain their immunomodulatory functions. Inhibition of Rap1 resulted in immunomodulatory impairment of MSCs.

Session No.: S12-01 Keynote Speaker

Clinical Application and Bone Regeneration by Octacalcium Phosphate and Collagen Composites

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The restoration of critical-sized bone defects is a crucial problem in oral and maxillofacial surgery, and autologous bone grafting has been a gold standard to restore them, whereas various biomaterials have been already applied clinically. Octacalcium phosphate ($Ca_8H_2(PO_4)_6 \cdot 5H_2O$; OCP) which has been suggested to be a precursor of biological apatite crystals in bones, and induced osteoblastic cell differentiation. Synthetic OCP granules possesses excellent bone regenerative properties more than hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP). However, OCP cannot mold by a sintering process, and the inherent brittleness precludes clinical application of OCP. Therefore, OCP and collagen composite (OCP/Col) has developed by mixing the synthetic OCP granules and medical-grade pepsin-digested atelocollagen isolated from the porcine dermis. Although the sponge of OCP/Col was easily compressed by hand, OCP/Col supplied suitable environment for bone regeneration to the invaded cells. Consequently, newly bone was formed in the sponge, and OCP/Col itself was resorbed. In the preclinical studies, OCP/Col significantly enhances bone regeneration more than OCP per se, β-TCP collagen composite, and HA collagen composite. Also, OCP/Col promptly enhanced bone regeneration without cell transplantation and exogenous osteogenic cytokines, and it was associated with active structural bone reconstitution. Then, an investigator initiated clinical trial of OCP/Col was performed for tooth extraction socket and cyst hole after approving by local institutional review board. After implantation of OCP/Col, postoperative course was stable and noticeable adverse event and laboratory disorders were not observed, and radiopaque figure was chronologically increased in the OCP/Col implanted defect, whereas OCP/Col itself had little radiopacity. These results suggest that OCP/Col would be a useful bone substitute. Recently, the sponsor-initiated multicenter clinical trial for the bone defects of oral and maxillofacial region was begun from April 2015, and it was aimed to commercialization of OCP/Col as a bone regenerative material.

Session No.: S12-02 Keynote Speaker

3D-printing of Complex-structured Bioscaffolds for Bone Therapy and Regeneration

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For therapy and regeneration of bone defects resulting from malignant bone disease, it is of great importance to develop multifunctional biomaterials for bone therapy and regeneration. Conventional biomaterials always lack multifunctional properties, limiting their application for treating and repairing bone disease (e.g. bone tumors)-initiated defects. How to design and prepare bioscaffolds with favorable microenvironments for disease therapy and tissue regeneration is one of interesting topics in the fields of biomaterials and tissue engineering. We developed several strategies, including harnessing nutrient elements, biomimetic structure and functional interface as well as thermo-therapy to construct multifunctional scaffolds by 3D-Pringting method for therapy and regeneration of bone tissues. It is interesting to find that both nutrient elements and biomimetic structure of the printed bioscaffolds have important effect on the stimulation of osteogenesis and angiogenesis of stem cells, and thermotherapy plays an important role to treating bone tumors. Therefore, we put forward new concept that 3D-Printed bioscaffolds combined bone therapy and regeneration could be a new direction of bone tissue engineering.

Session No.: S12-03 Invited Speaker

Preparation of Chitosan-based Nanofibrous Mats for Bone Tissue Engineering Applications

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It is known that nanofibers fabricated by electrospinning are structurally similar to the extracellular matrix. Thus they are regarded as potential materials for tissue engineering applications. In our laboratory, electrospun hybrid nanofibers containing chitosan (C), gelatin (G) polyvinyl alcohol (P) and gum arabic (A) were fabricated. To prepare hybrid solutions for electrospinning, the four constituents (C, G, P, A) were dissolved in an acetic acid aqueous solution instead of a toxic and highly corrosive solvent. The use of gum arabic could increase the amounts of chitosan (for providing tensile strength) and gelatin (for improving cytocompatibility) used in hybrid solution because the presence of gum arabic decreased the viscosity of the hybrid solution, thus reducing the amount of polyvinyl alcohol needed in electrospinning. The composition of the C/G/P/A hybrid solution and the process parameters of electrospinning were optimized. The properties of the C/G/P/A hybrid nanofibrous mats including microstructure, thermal properties, stability in aqueous solution, mechanical properties, cytocompatibility and osteogenic capability were also analyzed. We found that when using a moderate concentration of acetic acid (20 wt%) as solvent, C/G/A hybrid nanofibers with a weight ratio of 8:8:0.5 (C8G8A0.5) could be produced by electrspinning. When a small amount of polyvinyl alcohol was added (2 wt%), electrospun C/G/P/A (C8G8P2A0.5) nanofibers could be fabricated and collected more steadily. After crosslinked by glutaraldehyde vapor, the stability of nanofibers in PBS buffer was improved. The tensile strength of the C/G/P/A nanofibrous mats was 2.53±0.22 MPa after 12 hour crosslinking. The cytocompatibility and osteogenic differentiation of mesenchymal stem cells on the C/G/P/A mats were also characterized. The cells exhibited favorable proliferation, high ALP activity, elevated osteocalcin expression and mineralization under osteoinductive conditions, suggesting that the C/G/P/A hybrid nanofibrous mats may be utilized for bone-related tissue engineering applications. (supported by MOST 104-2221-E-002-174)

Session No.: S12-04

Investigation of Synergistic Effects of Inductive and Conductive Factors in Gelatin-based Cryogels for Bone Tissue Engineering

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Macroporous and biocompatible scaffolds for bone tissue engineering were prepared from 4% gelatin (G) and 4% gelatin/2% nanohydroxyapatite (nHAP), (GN), by cryogelation. The cryogels have interconnected pores with pore size around 100 mm and a high degree of cross-linking. The incorporation of nHAP slightly reduced the porosity, degree of crosslinking, swelling kinetics and equilibrium water uptake, but enhanced the toughness of the cryogel scaffolds. The osteo-regeneration potential of GN cryogels was further enhanced by binding with bone morphogenetic protein (BMP-2) to produce the gelatin/nHAP/BMP-2 (GNB) scaffold. The efficacy of BMP-2 incorporation was tested through in vitro release studies and a sustained release profile could be observed from the cumulative BMP-2 release curve. To elucidate the effect of cryogel composition on cell proliferation and differentiation, rabbit adipose-derived stem cells (ADSCs) were seeded in cryogel scaffolds. In vitro studies demonstrated a reduced proliferation rate and enhanced osteogenic differentiation of ADSCs in GNB cryogel scaffolds from the combined effect of nHAP and BMP-2, judging from the elevated alkaline phosphatase activity and the degree of mineralization. Confocal microscopy confirmed high viability and good cytoskeletal spreading of ADSCs on cryogels while osteocalcin protein quantification affirmed the dominance of GNB in the osteogenic differentiation of ADSCs compared to G and GN cryogels. The maximum osteogenesis capability of GNB was also confirmed through the up-regulation of specific bone maker genes of early marker protein collagen I and late marker protein osteopontin. From an in vivo animal model, computed tomography analysis confirmed the superior bone regeneration capability of ADSCs in GNB cryogels by implanting ADSCs/GNB cryogel constructs in rabbit calvarial critical size defects. Taken together, the results demonstrate that G cryogels modified with osteo-conductive nHAP and osteo-inductive BMP-2 could provide cues to synergistically promote the osteogenesis of ADSCs in vitro and in vivo.

Session No.: S12-05

Wharton's Jelly Stem Cell as an Allogenic Cell Source for Bone Tissue Engineering - An in Vivo Safety Study

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Introduction: Wharton's Jelly stem cells (WJSCs) have been used as alternative to bone marrow derived mesenchymal stem cells (BMMSCs). We investigated the population of MSCs derived from different segments of human umbilical cord - the maternal, middle and fetal segments. MSCs were successfully expanded from three centimetre-long segment. MSCs from maternal and fetal segments were preferred in terms of their growth profile, cell viability, pluripotent embryonic markers expression, and higher osteogenic potential in vitro compared to those derived from the middle segment. We proceeded to form tissue engineered bone constructs using osteo-differentiated MSCs from maternal and fetal segments. We then investigated the safety and efficacy of transplanting these xenogenic bone constructs in immunocompetent mice (BALB/C). Methods: Animals were divided into 4 groups (control, maternal, fetal and fibrin group). 3D bone construct were formed with human fibrin and WJSCs from maternal and fetal segments. After one month of implantation, mice were euthanized. Bone constructs, lymphoid organs were harvested, fixed and stained with haematoxylin and eosin. Serum samples were obtained for inflammatory markers detection. Results & Discussion: Mineralized bones were found in constructs of all except the fibrin group. Xenogenic fibrin had triggered an acute reaction evident by the enlargement of thymus and spleen in fibrin group. Maternal group showed the least immunogenic response based on the minimal changes seen in lymphoid organs. Pro-inflammatory cytokines (MMP-3, ICAM-1, galectin-3, fractakine, TNFα, IFNγ, IL-3, IL-6 and IL-1α) were highest in fibrin group. Anti-inflammatory cytokines (HGF, galectin-1, IL-13 and IL-10) were upregulated in fetal and maternal groups. Conclusion: Xenogenic WJSCs from maternal and fetal segments have the ability to suppress acute reaction toward fibrin. Bone constructs using WJMSCs from maternal segments are safe to be used allogenically for bone regeneration. These results were consistent with our previous findings in lymphocyte proliferation assay in vitro.

Session No.: S12-06

Low-dose Rhbmp-2 Onto Coral Scaffold Mixed with Autologous Mscs-seeded Coral-based Tissue Engineering Constructs to Repair Large Segmental Bone Defects

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INTRODUCTION Tissue engineering constructs (TECs) combining calcium-based scaffolds and mesenchymal stem cells (MSCs) have given promising results in bone regeneration. However, results remain inconsistent. Lack of osteoinductive potential is a critical issue. Thus, adjonction of osteoinductive factors might enhance bone formation. RhBMP-2 is the most osteoinductive factor. But supraphysiological dosages are currently used and led to adverse effects. The objective of the study was to evaluate mixed-TECs combining coral, autologous MSCs and low-dose rh-BMP-2 in a clinically relevant model. MATERIALS AND METHODS MSCs were isolated from bone marrow and seeded onto Acropora coral scaffolds in granular form. RhBMP-2, at different doses (low: 68µg/defect; high: 680µg/defect), was absorbed onto coral. Adsorption and bioactivity were accessed by UV and C2C12 culture. Twenty-three sheep were used. Metatarsal large bone defects (25 mm) were performed and stabilized by plate. Defects were filled with scaffolds loaded with: (i)MSCs/low-dose rhBMP-2 (1; n=6), (ii)low-dose rhBMP-2 (2; n=5), (iii)high-dose rhBMP-2 (3; n=5), (iv)MSCs (4; n=7). Radiographic follow-up was performed until 4 months. Bone formation and scaffold resorption were assessed by micro-CT and histomorphometry. RESULTS Adsorption rate was 65±6% with rhBMP-2 kept bioactive. Bone formation did not differ quantitatively between groups. However, bone union was more frequent in group 1 (3/4 united-cortical in 3/6, 1/5, 2/5 and 2/7 sheep in groups 1 to 4, respectively). Bone tissue appeared normal. Coral resorption (almost complete) did not differ between groups. DISCUSSION AND CONCLUSIONS Coral appeared to be a good carrier for rhBMP-2. There seemed to be a benefit to associate low dose rh-BMP-2 coral-based TECs with MSCs-seeded TECs, as this strategy allowed an increase of bone unions in our model. Yet, results remain inconsistent. Higher rhBMP-2 dosages did not led to adverse effect. There should be a greater effect when increasing rhBMP-2 dose, which remains far lower than ones currently used.

Session No.: S12-07

The Use of Demineralized Bone Matrix (DBM) Based Implantable and Biomimetic Microcarrier for Large Scale Stem Cell Expansion and One-step Tissue Engineered Bone Graft Construction

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Tissue engineered bone grafts (TEBG) using mesenchymal stem cells (MSCs) demonstrate great potential for large bone defect treatment. However, the current MSC expansion technique and the multi-step TEBG construction strategy are rather complicated and required repeated trypsinization, which can lead to the rapid cellular aging and compromised osteogenesis; this has considerably hampered the further clinical translation of bone tissue engineering (BTE) technology. Microcarriers may be useful for addressing this problem, but many of the currently available microcarriers are not biodegradable and osteoconductive, thus unsuitable for in vivo BTE therapeutic application. In current study, we developed an implantable demineralized bone matrix based microcarrier (DBM-MC) for efficient MSC expansion; furthermore, we established an DBM-MC based integrated TEBG fabrication strategy to seamlessly integrate the multiple procedures including cell seeding, expansion, and osteogenic priming under a continuous dynamic rotation condition. When benchmarked with Cyt y, and supported efficient cell adhesion and fast cell proliferation with the MSC characteristics well maintained. However, when expanded in vitro and implanted in vivo ectopically, the MSC mediated DBM-MC constructsachieved much more new bone formationwith well vascularization throughout the dense bone tissue; whereas, MSC mediated Cytodex 3 constructs experienced limited bone formation with scarce vascular network and empty necrotic cavities in the core region. Moreover, the μTEBG constructs generated via this integrated system was used for critical sized cranial defect treatment and achieved successful defect bridging at 3 months with two folds more bone regeneration. The implantable DBM-MC based integrated system can provide an enclosed, large-scale, less trypsinization, semi-automatic and single-step fabrication process to generate highly effective μTEBGs with outstanding osteogenic and angiogenic efficacy, thus demonstrating great potential for the BTE application in clinics.

Session No.: S13-01 Keynote Speaker

Chondrocyte Differentiation and Related Signal Transduction by Hydrostatic Pressure Loading

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Articular cartilage is focused as one of the most interest tissue to be regenerated in the field of regenerative medicine, because many elder people suffer from osteoarthritis (OA), which is of the major diseases in cartilage. Articular cartilages have adequate visco-elastic mechanical properties for sustaining weight and movements. However, it is still difficult to regenerate articular cartilages, which have enough sizes and adequate mechanical properties. When articular cartilage is loaded with compressive stress, then chondrocytes are known to be loaded with hydrostatic pressure up to about 10 MPa. Therefore, we have focused on how chondrocytes sense the hydrostatic pressure, how intracellular signals and gene expressions are regulated in terms of chondrocyte differentiation and cartilage regeneration. Membrane mechanical properties are thought to be critical in modulating cell signaling. The plasma membrane is predominantly composed of lipids, cholesterol and proteins, and its fluidity is tightly regulated by cholesterol and lipid desaturases. Hydrostatic pressure is known to affect membrane fluidity in microorganisms. To determine whether membrane fluidity was also altered in mammalian cells under pressure, we investigated the effects of pressure on membrane fluidity and desaturase expression in mouse chondrogenic ATDC5 cells. A fluorescent probe Laurdan imaging of pressurized ATDC5 cells showed a linear increase in the Laurdan GP (Generalized Polarization), and hence a decrease in fluidity, under increasing pressure. PCR analyses showed that ATDC5 cells under 10 or 20 MPa for 24h showed a significant decrease in the expression of all four desaturases under study. Cholesterol, which also decreased membrane fluidity, had a similar effect but removing cholesterol from the plasma membrane did not affect the desaturase down-regulation under pressure. These results show that hydrostatic pressure affects chondrocyte plasma membrane properties and suggest that hydrostatic pressure loading could interact with intracellular signal transduction via changes in plasma membrane fluidity.

Session No.: S13-02 Keynote Speaker

Extracorporeal Shockwave Therapy Enhances Angiogenesis in Early Hip Necrosis

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Purpose: This study investigated the systemic and local effects of different dosages of extracorporeal shockwave therapy (ESWT) in early osteonecrosis of the femoral head (ONFH).

Materials and Methods: Thirty-three patients (42 hips) were randomly divided into three groups. Group A (10 patients with 16 hips) received 2000 impulses of ESWT at 24 Kv to the affected hip. Group B (11 patients with 14 hips) and Group C (12 patients with 12 hips) received 4000 and 6000 impulses of ESWT respectively. The evaluations included clinical assessment, radiographs, MRI, microcirculation (Ktran) and plasma volume (Vp), and serum biomarkers (NO3, VEGF, BMP-2, osteocalcin, TNF-α, IL-6, substance P, CGRP, DKK-1 and IGF).

Results: Significant differences of pain and Harris scores were noticed between Group A and C from 6 to 24 months after treatment (all $P<0.05$). Total hip arthroplasty was performed in 3 patients (4 hips) in Group A, but none in Groups B and C. Group C showed significant changes in serum angiogenesis, osteogenesis, anti-inflammation, pain threshold and tissue regeneration biomarkers within one month after treatment (all $P<0.05$). No significant changes in the infarction volume were noted in all groups (all $P > 0.05$). The post-treatment Ktran and Vp in the peri-necrotic areas of Group B and C were significantly greater than pre-treatment Ktran and Vp (both $P<0.05$).

Discussion: Prior study reported ESWT is effective in early ONFH. However, the optional dosage is unknown. The results of the study revealed ESWT enhances angiogenesis at peri-necrotic areas of the femoral head that may in turn, improve subchondral bone remodeling and prevent femoral head collapse.

Conclusions: High dosage ESWT is more effective in early stage ONFH. The systemic beneficial effects of ESWT may ultimately enhance microcirculation of perinecrotic areas and prevent femoral head collapse.

Session No.: S13-03 Invited Speaker

Hepatocytes Cultures in Stretch and Relax Model

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Liver tissue engineering has been increasingly important in recent years with many applications such as providing alternative therapies to liver diseases, providing a bridge to liver transplantation and acting as an in vivo model for drug metabolism and toxicity studies. Numerous experiments have demonstrated that culture configurations such as the collagen sandwich configuration, three-dimensional spheroid culture and co-culturing can aid in the restoration of hepatocyte polarity and maintain their liver-specific functions. However, isolated hepatocytes cultured in these methods still suffer from limited repolarization. In this study, we hypothesized that the application of compressive forces would maintain hepatocyte polarity for longer period of culture. In our cell culture system, rat hepatocytes were cultured on a stretchable polydimethylsiloxane (PDMS) substrate subjected to a uniform stretch and subsequently relaxed after 24 hours. These relaxation resulted in the compression of cells in the culture. The hepatocytes were cultivated for 4 days before being fixed to study their functional polarity. Our results indicated that all stretched cultures had better hepatocytes repolarization when compared to the unstretched controls. At day 4, the 10% stretched cultures had better repolarization. Moreover, we also established proof-of-concept of the cell-stretcher in the effective application of uniaxial compressive forces through an ergonomic and easy-to-use design. Hepatocytes cultured in our novel culture system could potentially be used for long-term metabolic and drug toxicity studies but also for the more accurate modelling of in vivo tissues.

Session No.: S13-04 Invited Speaker

Surface Modification with Laser Ablation for the Study of Cell Contact Guidance Behavior in Skin and Vasculature Regeneration

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In the field of tissue engineering, the choice of scaffolds had been proven one of the most important factors for success in cell regeneration. Correct choice of scaffold not only promotes cell adhesion, but also facilitates cell differentiation and elongation. Part of this influence was identified as contact guidance, which described the cell alignment induced by micro- and nano-surface topography. To understand this phenomenon, micro- and nano-patterned scaffolds with high precision were required. Over the past 20 years, high precision patterning of polymeric materials were largely performed via the application of microelectromechanical systems (MEMS) and miniaturization technologies. This technology have enabled the creation of biomedical microimplants for the treatment of various chronic and acute illnesses. Although MEMS-Fab is a technology with great precision, it is extremely expensive and time consuming, especially for prototyping. In this work, biodegradable polymeric materials were synthesized with high mechanical strength, flexibility, optical transparency, and biocompatibility. Micropatterns were created via a novel laser ablation microfabrication method. Micropatterns were ablated on two recently developed classes of biodegradable elastomeric materials, poly(glycerol sebacate)s (PGS) and poly(1,3-diamino-2-hydroxypropane-co-polyol sebacate)s (APS), showing highly tunable in vivo degradation half-life comparing to many other commonly used biodegradable polymer. The novel fabrication technique using laser ablation is also reported with micro- and nano-patterning capabilities. Through laser ablation, different cell growth behavior was observed, and different micropatterns were identified for each type of cells. The fabrication process is fast, inexpensive, reproducible, and scalable, making the approach ideal for both rapid prototyping and study of cell contact guidance toward skin and vasculature regeneration.

Session No.: S13-05

The Effect of Anisotropic Strain and Flow Induced Shear Stress Combinations on Endothelial Cell Alignment

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INTRODUCTION: Cyclic strain and fluid flow are well known to affect cell behaviour. Also, isotropic and anisotropic strain can affect cells differently. While in-vivo cells experience varying degrees of anisotropy (d.o.a.), in-vitro anisotropic strain studies have mostly focused on uniaxial strains. In this study, we determined the effects of varying d.o.a., in combination with fluid flow shear stresses, on human umbilical vein endothelial cells (HUVECs) using a newly developed device. METHODS: The device has 100 units producing various anisotropic strains. This is achieved by stretching a polydimethylsiloxane (PDMS) membrane over circular pillars into surrounding ellipse trenches. The dimensions of the ellipse determine the d.o.a., which is defined as the ratio of maximum to minimum principal surface strains. The presence of fluid flow channels at varying angels to the ellipses allows for the determination of combined effects of anisotropic strains and flow induced shear stresses. HUVECs were mechanically stimulated. Simultaneously, cells were subjected to fluid flow shear stresses of ~5 dyne/cm2. The cells were fixed after 24 hours and stained with Alexa 488 Phalloidin and DAPI. RESULTS: Models and empirical measurements showed that strains with varying d.o.a. could be generated on the device. HUVECs aligned along the minimum principal strain direction when only strain was applied. An increase in d.o.a. resulted in increased cell alignment. Cells aligned along the flow direction when only flow was applied. When flow and strain were combined, alignment was predominantly in the direction of the flow, but an offset towards the minimum principal strain direction was detected. DISCUSSION & CONCLUSIONS: HUVECs respond to various d.o.a. The variations in response of cells highlight the need to study the effects of strains of varying d.o.a. on cells. Our device permits such experiments with an increased throughput, which makes it an important tool to better understand these mechanobiological principles.

Session No.: S13-06

Development of Novel Bioreactor System with Pulsatile Pressurization for Organ Fabrication

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Recently, numerous studies have demonstrated the feasibility of developing tissue perfusion systems in vitro for tissue engineering. We have previously reported successful fabrication of thick rat cardiac tissue with capillary structure using cell sheet-based tissue engineering and perfusion culture system. However, long term maintenance of an efficient flow rate has been challenging. Because sustainable long term perfusion and angiogenesis are vital for tissue fabrication, we attempted to mimic the mechanical stress found in vivo, ex vivo by creating a pressurized system. First, we constructed a bioreactor which has a pressure chamber, an infusion pump, a scale connected to artery and vein, and a gas mixer for pressurizing. Next, we confirmed pressurized conditions by comparing perfusion ratios. We tested 2 conditions; constant-pressurization (CP) and pulsatile-pressurization (PP) which mimics pressure change from body moving compared with non-pressurization (NP). The tissue used for perfusion was harvested from femoral artery and vein from 8 week-old LEW rat, and connected to the developed bioreactor. The perfusion with constant pressurization at 10 mmHg did not increase perfusion ratio when compared to NP one. However, in the case of PP which allow imitation of body movement, the perfusion ratio from 0-10 mmHg was significantly higher than NP group (55.1±2.1% versus 32.5±9.3% at 4-day, 52.1±3.0% versus 26.2±8.3% at 5-day, $p<0.05$, $n=3$). To verify the effect of pressurization on the tissue, bioluminescence activity assay and histological analysis of perfused tissue were carried out. After 14-days of perfusion, the PP group maintained high bioluminescence indicating viability and reduction of cell death in the tissue. In conclusion, we have developed a novel bioreactor system which can perform pulsatile pressurization during perfusion which improves perfusion ratio and maintains long term tissue viability.

Session No.: S13-07

The Effects of Eswt on Shoulder Stiffness. A Prospective Randomized Control Study

Jih-Yang Ko[1], Feng-Sheng Wang[1], Ching-Jen Wang[1], Wen-Yi Chou[1], Ka-Kit Siu[1], Feng-Sheng Wang[1], Ching-Jen Wang[1], Wen-Yi Chou[1], Ka-Kit Siu[1]

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[Introduction] In our-previous study of shoulder stiffness, we found that patients with shoulder stiffness had increased IL-1β expression in lesion tissue. Our recent data also showed anti-inflammatory effect of ESWT. We hypothesized that ESWT might be beneficial for rotator cuff lesions with shoulder stiffness. [Material and Method] We performed a prospective randomized double-blind study. The criterion for shoulder stiffness was ≥50% loss of passive range of motion (ROM). Patients were randomly divided to receive either shockwave or conservative treatment (control group) based on statistical randomization. In the shockwave group, we used OrthospecTM Extracorporeal Shock Wave Therapy 3000 impulse 24KV (0.32mJ/mm²) focus at two points at an area 1cm proximal to the insertion of supraspinatus. The sham treatment entailed use of the device in which the generator was disconnected. The treated area was inspected for local swelling, ecchymosis, or hematoma. VAS and Constant score were used for measurement. [Results] 21 patients were enrolled. Three patients underwent surgery 4 weeks, 3 months and 6 months after study respectively, due to severe symptoms. At the latest follow-up, 10 patients had sum of ROM >= 300°. At 6 months after treatment, the ESWT group had significantly better ROM and Constant score when compared with control group. [Discussion] Although ROM and symptoms improved in both groups, the ESWT group had better improvement than the control group 6 months after treatment. This phenomenon may be attributable to the physiological effects of ESWT and the slow progressive nature of shoulder stiffness. [Conclusion] ESWT may be beneficial for shoulder stiffness associated with rotator cuff lesions.

Session No.: S14-01 Keynote Speaker

Human Derived Biomaterials and Cells for Tissue Regeneration

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Plasma derived fibrin matrix is one of the most versatile biomaterials for tissue engineering and regenerative medicine. Being involved in a > 30 year development we can demonstrate advantages and limitations, application techniques and its special use for growth factor and cell delivery as well as a gene activated matrix. Another goal in our group is to use „medical garbage “for regenerative purposes. Therefore we use placenta derived materials (PD) – substances (e.g. collagen) and PD structures (e.g. amnion decellularized vasculature) as well as PD cells (e.g. amnion MSC). “Living” Amnion is used either directly (cryopreserved) using a clinically approved process, e.g. for wound healing and antifibrosis or in a new process that the stem cells residing on and in amnion (“sessile” cells) are differentiated in toto (osteo, chondrogenic direction). In addition we use cells from liposuction and from umbilical cord. Isolated stem cells are cultured with platelet derived factors (from outdated platelets) to avoid animal products and used directly or pre-differentiated, in autologous or allogeneic fashion. This talk should give an overview about the use of above mentioned procedures within the Austrian Cluster for Tissue Regeneration.

Session No.: S14-02

Production of a Contractile Muscle Tissue Construct Based on Cell Sheet Technology

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A technique for mimicking microstructures in native tissues is important to achieve production of biomimetic functional tissues. Particularly in mature skeletal muscle, the muscle fibers are highly oriented and the well-organized structure is essential to produce its mechanical functions. To control orientation of muscle cells, in this study a micropatterned thermoresponsive substrate have been developed. Since human skeletal muscle myoblasts can recognize the difference in cell-surface interactions between the stripe patterns of two different polymers, they were finally aligned on the surface with the direction of the micropatterns. In addition, due to the thermally induced surface alternation, they can be harvested as a single continuous cell sheet from the surface by lowering culture temperature to 20 ℃. In this study, the myoblast sheet was transferred onto a collagen gel. After 3 weeks of incubation in a differentiation medium, the aligned myoblasts differentiated into myotubes while maintaining their aligned orientation on the gel. Furthermore, it was observed that the myotubes contracted significantly by electrical stimulation (10V, 10 msec). Importantly, the contractile direction was regulated because of the well-organized myotube orientation in the tissue construct. Since the movement of contracting muscle can be tracked by image analyzing, response of the tissue construct against drugs was able to be evaluated by their contraction. For example, under the electrical stimulation condition, the muscle contraction was suppressed by the addition of ryanodine, and the effect of this drug was dependent on its concentration (10-100 uM). These results indicate that this muscle tissue construct has a potential as a tissue model for muscle disease drug discovery.

Session No.: S14-03

Preparation of Porous Poly-L-Lactic Acid Particles for the Development of Soft Tissue Fillers

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The external force or diseases made soft tissue defects, it not only lost normal function of the skin, but also affected the psychological trauma. It need the good therapy to fix defects of subcutaneous soft tissue. The stimulatory fillers (poly-L-lactic acid: PLLA), a biodegradable synthetic polymer, induced human fibroblasts to make collagen I synthesis and had a lasting effect than the replacement fillers like collagen or hyaluronic acid for subcutaneous volume restoration. They became a good choice of the clinically fillers to fix atrophy. However, there were still some clinical problems of the PLLA-based filler such as size, morphology irregular of the PLLA particles led to stimulate unevenly on atrophied positions then caused the granulation tissue swelling and papules. Thus the purpose of this study was to prepare even size and morphology of PLLA particles to improve the clinical problems. The PLLA was used to fabricate porous or nonporous microspheres through a modified double emulsion solvent evaporation parameters and analyzed biocompatibility, gene expression on different group of size and morphology of the PLLA particles to optimize the stimulatory fillers. We regulated stirring speed and different volume ratio between the PLLA and surfactant solutions to produce even sizes and increased the yield of the 40-100um porous PLLA particles. Each groups of PLLA particles had good biocompatibility and the 40-100um porous PLLA particles can reduce the degradation of collagen gene MMP1 expression in vitro which indicates that the controllable porous structure are of great potential as the stimulatory filler for subcutaneous application.

Session No.: S14-04

Platelet Lysate Suppresses the Inflammatory Response of Endothelial Cells by Inhibiting NF-κB Activation while Induces Proliferation of Quiescent Cells

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INTRODUCTION: Platelet Lysate (PL) contains a cocktail of growth factors and cytokines, which actively participates in tissue repair. In previous publications, we have shown that PL transiently increases the inflammatory response occurring in wound while induces proliferation of resident cells in keratinocyte and osteoblast systems. The aim of this study was to assess the activity of PL on endothelial cells, the first cells that respond to the wound, causing damage of blood vessels and resulting in the cascade of coagulation and release of platelet content. METHODS: Human Umbilical Vein Endothelial Cells (HUVEC) were obtained from primary cultures derived from fresh umbilical cords and cultured on gelatin coated plates in M199 medium supplemented with 10% FCS, 10 µg/l aFGF, 10 µg/L bFGF, 10 µg/l EGF, 1 mg/L hydrocortisone. PL was produced from platelet rich plasma obtained from a pool of human blood donors. Platelets were washed and re-suspended in phosphate-buffered saline at a concentration of 10 millions platelets/µl and subjected to 3 freeze/thaw cycles followed by a high-speed centrifugation. The supernatant, was added to complete culture medium at a final concentration as 5%. RESULTS: PL inhibits the inflammatory response induced by IL-1 by inhibiting the NF-κB activation and repressing the secretion of IL-6 and IL-8. PL increases the proliferation of endothelial cells and induces proliferation in quiescent cells by activating the pathways of ERKs and AKT, and the synthesis of Cyclin D1. CONCLUSIONS: PL has a protective activity towards endothelial cells by inhibiting the response to inflammatory cytokines possibly released in vessels damage. PL is able to induce in quiescent cells a proliferative response, important for vessel repair indicating a possible mechanism in the restoration of blood vessel functional activity in wound healing.

Session No.: S14-05

Nasal Fibroblast Secretome Analysis for Identification of Factors That Accelerate Respiratory Epithelial Cells Wound Healing

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Cultured nasal fibroblast secretes growth factors, cytokines and extracellular matrix proteins. These secretory protein accelerates proliferation and increase cell migration, thus could improve airways wound healing. The aim of this study was to identify the factors produced by nasal fibroblast conditioned medium that accelerate in vitro wound healing of respiratory epithelial cells (RECs). Nasal turbinates were obtained from consented patients undergoing turbinectomy procedures. RECs and fibroblasts were co-cultured using Defined Keratinocytes Serum Free Medium (DKSFM) and F-12 :Dulbecco's Modified Eagle's Medium (1:1) supplemented with fetal bovine serum. Fibroblasts were differentially trypsinized, leaving the colonies of RECs to reach confluency. The conditioned medium was collected by culturing fibroblast either in DKSFM or serum free F12: DMEM (1:1), denoted as NFCM_DKSFM and NFCM_FD, respectively. SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis) and 1D nanoLC ESI MS/MS analysis by Triple TOF were performed to investigate the differentially expressed proteins in both conditioned medium. The LC/MS/MS data were searched using Mascot (Matrix Science) against the Swissprot Human Database and protein identification was analyzed by PANTHER classification system. SDS-PAGE detected presence of many secreted proteins in fibroblast-conditioned medium, which were further confirmed by LC/MS/MS results. Analysis by LC/MS/MS showed a total of 182 unique proteins, which 146 proteins in NFCM_DKSFM and 116 proteins in NFCM_FD, respectively. Potential protein class identified by PANTHER classification system are mostly involved in receptor, extracellular matrix protein, enzyme modulator, cell junction protein, cell adhesion molecule, calcium binding protein and signalling molecule. In conclusion, it is predicted that various growth factor families and cytokines that are present in the secretome were involved in promoting wound healing, proliferation and migration of RECs.

Session No.: S14-06

Restoration of Articular Cartilage Defect with Kartigen®

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Repairing articular cartilage defect becomes a hot topic recently with such lesion is not a few. Scaffold along (TruFit Plug) or procedure along (microfracture) do not restore a “solid” articular surface. Using cells to restore the defect is the “main stream” to achieve hyaline cartilage surface. In the last years, Kartigen® , an immature chondrocytes (derived from mesenchymal stem cells,), has been developed to restore the cartilage defects. Twelve participants were enrolled in the pilot clinical trial. The pre- and post-OP evaluations, including X-ray, MRI, arthroscopy, histological analysis and IKDC assessment were done and showed encouraging results in follow up of 5 to 6 years. Biopsies of implanted tissue revealed the presence of glycosaminoglycan and type II collagen. Neither valgus osteotomy nor other operative procedures were performed in the treatment. No complications such as deep vein thrombosis, infection or tumor formation were noted. An even distribution and integration of implanted tissue was achieved with the phenotypic expression of hyaline cartilage. The engineered bioproduct seems effective in repairing full-thickness chondral defects of the knee.

Session No.: S15-01 Keynote Speaker

3D Printing Approaches for Generating Complex Tissue Engineered Mimetics

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3D printing has risen in popularity in the tissue engineering and regenerative medicine field as a result of its ability to fabricate complex tissues consisting of unique shapes, microstructures, and strand patterns. Aiming to improve the materials and processing techniques in 3D printing, our laboratory has focused on the development of engineered constructs to recapitulate native tissue structure and provide advanced platforms for tissue growth. To capture the complexity of different tissues, our lab utilizes stereolithography-based and extrusion-based additive manufacturing. Herein, we have generated patient-specific vascular grafts, prevascular networks for bone tissue engineering, dermal dressings, multi-phase cartilaginous scaffolds, and developed a cell-laden placenta model in order to better address clinically relevant needs such as heart disease, osteochondral defect repair, and preeclampsia. Based on the specific application, our choice of materials varies from synthetic polymers with tunable properties to UV crosslinkable naturally derived hydrogels. Being aware that providing adequate nutrient and waste exchange in the development of large tissues is a key challenge, we have also developed 3D printed perfusion bioreactor chambers using an acrylate photocurable resin to enable specific cell surface shear stresses, to enhance mesenchymal stem cell (MSC) proliferation and differentiation. This presentation will cover 3D printing approaches to generate complex structures developed in our laboratory and their potential to solve relevant, emerging problems in tissue engineering.

Session No.: S15-02 Keynote Speaker

Cell Encapsulation and Printing

Claudio Migliaresi[1], Volha Liaudanskaya[1], Devid Maniglio[1], Nicola Cagol[1], Antonella Motta[1]

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Cell Printing technology could provide an enabling platform for the fabrication of hierarchically structured and functional cell assemblies mimicking composition, structure and physiological behavior of animal tissues and namely human tissues. These assemblies could be used as part of tissues for tissue engineered replacements that could be implanted in humans starting for the patient's differentiated or stem cells. Moreover, they could constitute 3D model cell assemblies for the evaluation of drugs, contaminants, additives to food, pesticides, cosmetics etc., and also used as biological models to study the mechanism of in vitro induced diseases. The platform should comprise: 1. the selection, design, control and optimization of cells encapsulation materials and methods; 2. the design and implementation of a computer controlled cell printing machine; 3. the identification of specific dynamic culture conditions in specific bioreactors to drive cell differentiation, ECM production, tissue assemblies. The ideal path should proceed through the following steps: Encapsulation of cells under proper conditions and in proper gel materials; Deposition of the capsules of gel polymers containing different types of cells following specific 3D geometric patterns; Transfer of the printed assemblies to specifically designed dynamic bioreactors for culture under tailored dynamic conditions till the development of vascularized extracellular matrix. The present talk will explore different cell printing technologies and will outline some of the results that we have achieved in the field by using a Electrodynamic Spraying method and sodium alginate as an encapsulating matrix material. Considerations about the characteristics of the encapsulating materials and results about the effect of the method and of the materials on encapsulated cells viability and metabolic activity will be presented.

Session No.: S15-03 Keynote Speaker

3D Bioprinting for Tissue Engineering: Challenges and Opportunities

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3D Bio-Printing uses cells, biologics and/or biomaterials as building block to fabricate personalized 3D structures or functional in vitro biological models for regenerative medicine, disease study and drug discovery. This presentation will report recent advances on 3D Bioprinting. An overview of 3D Printing to Bio-3D Printing will be given according to its technological development and application stages. Engineering printing process will be reviewed. Examples of applying 3D Printing techniques to develop tissue engineering model, drug testing model and cancer tumor model will be given, along with discussions on challenges and opportunities in the field of 3D Bio-Printing.

Session No.: S15-04

3D-printed Atsttrin-incorporated Alginate/Hydroxyapatite Scaffold for Bone Defect Repair

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It is reported that under local or systemic inflammatory process in bone defect, high expression levels of pro-inflammatory tumor necrosis factor (TNF)-α can decelerate and impair bone formation and regeneration. However, there are few available scaffolds with anti-inflammatory function. Progranulin (PGRN) and its derived engineered protein, Atsttrin, were reported to antagonize TNF-α. In addition, PGRN was also reported to promote bone healing through interacting with TNF/TNFR signaling. However, whether Atsttrin-incorporated scaffold could facilitate bone healing via interacting with TNF/TNFR signaling has not been studied yet. In this study, a 3D bioprinting system was used to fabricate Atsttrin-incoporated alginate/ hydroxyapatite (3D-printed Atsttrin-Alg/nHAp) composite scaffolds and Atsttrin release from this 3D-printed scaffold was analyzed. Then the efficacy of this bioactive scaffold on bone regeneration was evaluated both in vitro and in vivo. This 3D-printed Atsttrin-Alg/nHAp scaffold presented precisely defined structure, could sustain Atsttrin release for at least 5 days, exhibited no cytotoxicity and supported cell adhesion. We also found that Atsttrin could reverse the suppressive effect of TNF-α on BMP-2-induced osteoblastic differentiation in vitro. When implanted into the site of post-calvarial defect, this 3D-printed Atsttrin-Alg/nHAp scaffold significantly reduced the number of TNF-α positive cells within wound sites 7 days after surgery. Moreover, histological staining and X-ray scanning results showed that this 3D-printed Atsttrin-Alg/nHAp scaffold enhanced bone regeneration in mice calvarial bone defect model at 8 and 16 weeks. In summary, this 3D-printed Atsttrin-Alg/nHAp scaffold with precise structure control and anti-inflammatory property could be adopted for bone defect repair .

Session No.: S15-05

Development of Bioresorbable Tissue Scaffolds Using a New Precision Multi-axial 3D Printing Technology

Qing Liu[1], Qinghua Zhao[1], Guixin Shi[1], Hugh Zhao[1], Jie Zhang[1]

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Three-dimensional (3D) printing technology has been widely used to make prototypes and tissue engineering scaffolds of various shapes and structures. However, when it comes to printing porous tubular structures, it is a time consuming process due to its layer by layer fabrication nature and additional steps needed for removing the supporting structures/materials from the scaffolds. Also, the poor surface finish and micro defects of the 3D printed scaffolds are the factors that will lead to early mechanical failure of the scaffolds when they are used in load bearing situations. The goal of this study was to develop a novel precision multi-axial 3D printing technology to produce porous tubular bioresorbable scaffolds for various applications. Using this patented precision multi-axial 3D printing technology, we have successfully developed vascular bioresorbable scaffold (BRS). The BRS functions to restore blood flow by providing a temporary mechanical support for keeping the diseased blood vessel open during the first several months post implantation. Unlike the currently used metallic stents, the BRS is gradually degraded and absorbed in the body without leaving any permanent foreign material behind as the treated vessel remodels and returns to its natural unrestrictive state. This patented 3D printing technology was capable of producing BRS within minutes in a single-step process by directly using raw material particles. Additionally, totally bioresorbable porous tubular polymer scaffolds for tracheal, urological, and esophageal treatment and repair were also produced to demonstrate the huge potential of this novel 3D printing technology.

Session No.: S15-06

Design and Validation of Flexible Open-source Printhead for 3D Bioprinting

Andres Sanz-Garcia[1], Enrique Sodupe-Ortega[2], Carmen Escobedo-Lucea[3]

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[3]University of Helsinki

Bioprinting is becoming a key technology to create layer-by-layer three-dimensional (3D) constructs using cells and biopolymers. While much research has been devoted to the bioinks, numerous challenges remain in bringing affordable bioprinters that can generate cell-laden scaffolds with accurate geometry. This has been traditionally a closed technology with a limited presence in laboratories due to the high acquisition costs. In order to continue with collaborative projects like Fab@Home, we present a novel open-source printhead with enough flexibility to be assembled in many machines. For 3, 5 and 10 mL syringe volumes, our device provides an accurate control of the temperature from 5 to 38 ℃ that allows finding the optimal printability of the bioinks. We used our system to create cell-laden constructs with different geometries using human adipose-derived mesenchymal stem cells (hASCs). Cells were encapsulated in various concentrations of blended bovine and porcine gelatine with alginate. Setting parameters were tuned for each specific bioink avoiding high shear during the printing process to assure a cell survival closed to 90% after CaCl2 cross-linking. Preliminary results of cell proliferation and gene expression after 7 days suggest differences but indicating that our approach is a particularly positive development. The combination of bioprinting and open-source solutions enables the expansion of this technology among tissue engineering and regenerative medicine laboratories and can potentially give a boost to accelerate its development.

Session No.: S15-07

Peptide Bioinks for Bioprinting 3D Hydrogel Scaffolds to Build Organotypic Tissue Constructs

Yihua Loo[1], Andrew Wan[1], Charlotte Hauser[2]

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[2]Kaust

With increasing social emphasis on animal welfare, there has been greater pressure to replace in vivo animal testing with in vitro assays. However, conventional assays typically utilize cells cultured on two-dimensional substrates which then fail to recapitulate the full complement of physiological functions. This is attributed to cell behavioral changes when taken out of their native three-dimensional (3D) environment. Furthermore, most organ systems typically comprise of multiple cell types arranged in a specific pattern for the optimal coordination of tissue response. 3D bioprinting is arguably the best technique to re-create organotypic constructs for high-throughput screening and regenerative medicine. The ability to rapidly, precisely and reproducibly deposit cells and bioactive moieties in a small area enables the design of complex constructs comprising of multiple cell types and microenvironments. The development of bioprinting technologies is hampered by the lack of suitable bioinks to serve as the structural scaffold. We report the first peptide bioinks – lysine-containing hexapeptides which self-assemble under physiological conditions into stable, nanofibrous three-dimensional hydrogels with unprecedented stiffness. These peptides are ideal bioinks as they (1) form scaffolds with adequate mechanical strength, rigidity and shape fidelity, (2) exhibit tuneable gelation to facilitate extrusion, (3) form scaffolds which resemble the native cellular microenvironment, (4) possess good biocompatibility in vitro and in vivo, (5) are amenable to chemical modifications to tailor the cellular microenvironment, and (6) can be synthesized on a commercial scale with minimal batch-to-batch variation. We have successfully encapsulated human pluripotent stem cells for long term culture with repeated passaging for up to 9 months, and also prepared organotypic gastrointestinal and skin models with multiple cell types localized in specific domains. These biological constructs can be potentially be applied organoid models for screening small molecules, studying cell behaviour and disease progression, as well as tissue-engineered implants for regenerative medicine.

Session No.: S16-01 Keynote Speaker

Microenvironment for Stem Cells

Barbara Chan[1]

[1]The University of Hong Kong

Many researchers wish to influence stem cell fate by manipulating their microenvironment. Although differentiation is the most frequently studied stem cell fate, other cellular fate processes including adhesion, migration, orientation and proliferation are also the targets of the microenvironment manipulation. Stem cells are known to sense and respond to multiple types of signals present in their microenvironment including soluble factors, neighbor cells, supporting matrix and mechanical loading. Various approaches to reconstitute these signals in different scaffolding systems during tissue engineering will be discussed. A few platform technologies including collagen microencapsulation (3D) and multiphoton-based protein micro-fabrication (2.5D) will be discussed. Examples in modifying the matrix and the mechanical microenvironment for stem cell fate manipulation will be given.

Session No.: S16-02 Invited Speaker

Scaffold-mediated Delivery of Small Non-coding Nucleic Acids to Direct Stem Cell Fate

Sing Yian Chew[1]

[1]Nanyang Technological University

Nanofiber substrates closely mimic of the size-scale and architecture of the natural extracellular matrix. These constructs represent a novel class of materials in regenerative medicine. Fibrous topographical cues can direct cellular response and stem cell fate. Combined with the incorporation of small non-coding RNAs, these substrates provide synergistic topographical and biochemical cues to seeded cells. Here, we will discuss our recent findings on the roles of nanofiber topography on host implant integration; nerve regeneration after spinal cord injury and gene silencing. The advantages of scaffold-mediated gene silencing in enhancing host-implant integration and directing stem cell neuronal differentiation and oligodendrocyte differentiation and myelination will also be presented.

Session No.: S16-03 Invited Speaker

Guidance of Stem Cell Function and Bone Regeneration Using Functionalized Fibrous Materials

Heungsoo Shin[1], Sajeesh Kumar Madhurakkat Perikamana[1], Jinkyu Lee[1]

[1]Hanyang University

Engineering bone regeneration has gained considerable interest in recent years due to various clinical issues and the limited availability of suitable bone grafts. At the earlier stages of bone healing, uncommitted mesenchymal stem cells migrate from the bone marrow to the wound area and are then differentiated into osteoblasts by a group of growth factors. Then, the secreted type I collagen became mineralized in a later stage by chemical interactions with calcium and phosphate ions in the microenvironment, providing the main structural framework of bone tissue. Therefore, it is of importance to consider engineering strategies to direct multi-facet processes in a bone microenvironment for regeneration of physiologically relevant bone tissue. In this presentation, how the guidance of in vitro stem cell function and in vivo bone formation can be affected by functionalized fibrous materials with capability of providing chemical and geometrical signals will be discussed. For example, osteo-inductive molecules (proteins or their putative peptides) were immobilized onto fibrous materials to form inductive microenvironment for osteogenic differentiation of stem cells. In addition, 3-dimensional architecture of fibrous materials was controlled to exhibit randomly or anisotropically aligned structure. We found that the structure of fibrous materials not only guided in vitro migration and adhesion of stem cells, but also regulated shape and assembly of in vivo formed collagen secreted from endogenously migrating stem cells. We also developed surface to control the gradient of chemical signals that can modulate spatial distribution of stem cells to recapitulate the interfacial bone tissue in native tissue where hard tissue is graded with soft tissue.

Session No.: S16-04 Invited Speaker

Engineering Emt Using 3D Microenvironment to Promote Hepatic Differentiation for Drug Hepatotoxicity Evaluation

Yanan Du[1], Jingyu Wang[1], Rui Yao[1]

[1]Tsinghua University

Directed differentiation of human embryonic stem cells (hESCs) towards hepatocyte-like cells holds great promise to provide alternative cell sources for drug metabolism/hepatotoxicity evaluation. However, efficiency of hepatic differentiation on planar tissue culture plates is usually low and not satisfactory. We have realized hepatic differentiation of hESCs in 3D configuration with better mimicry of embryonic liver development which produced more homogenous and mature hepatocyte-like cells with significantly lowered AFP expression and elevated hepatic functions compared with 2D counterpart. Meanwhile, epithelial-mesenchymal transition (EMT) was known to occur in 2D cultured hepatocytes accompanied by decreased hepatic functions. Therefore, we hypothesize that 3D microenvironment might enhance hepatic differentiation and functions through regulating the EMT status. To test the hypothesis, biomaterial-engineered EMT was achieved by culturing HepaRG, a hepatic progenitor, as 3D spheroids (SP-3D) or 3D stretched cells (ST-3D) in non-adherent and adherent micro-scaffold respectively. In SP-3D, constrained EMT of HepaRG, as represented by increased epithelial markers and decreased mesenchymal markers, was echoed by improved hepatic functions. To investigate the relationship between EMT status and hepatic functions, time-series RNA-Seq and gene network analysis were used for comparing different cell culture models, which identified histone deacetylases (HDACs) as key mediating factors. Protein analysis confirmed that high HDAC activity was correlated with high expression of Cadherin-1 (CDH1) and hepatic function genes, which were decreased upon HDAC inhibitor treatment in SP-3D, suggesting HDACs may play positive role in regulating EMT and hepatic functions. To illustrate the application of EMT-regulated 3D configuration in drug safety evaluation, hepatotoxicity and metabolism assays of two hepatotoxins (i.e. N-acetyl-p-aminophenol and Doxorubicin) were performed and SP-3D showed more biomimetic toxicity response, indicating regulation of EMT as a vital consideration in designing stem cell microenvironment especially in 3D configuration.

Session No.: S16-05 Invited Speaker

Microenvironmental Micro- and Nanotopography in Regulating Stem Cell Differentiation

Evelyn Yim[1], Soneela Ankam[2], Benjamin Kim Kiat Teo[2]

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The microenvironment of stem cell niche comprises of a rich combination of biochemical and biophysical cues. Regulated biophysical cues, such as nanotopography, have been shown to be integral for tissue regeneration and embryogenesis. Synthetic nanostructures have been demonstrated to be capable to drive specific cell differentiation but the sensing mechanism of nanocues remains poorly understood. We seek to understand the role of ECM, cytoskeletal contractility and integrin-activated focal adhesion kinase (FAK) in human mesenchymal stem cell (hMSC) and human embryonic stem cells (hESCs) mechanotransduction on nanotopography. On nanogratings, hMSCs developed aligned stress fibers and showed an upregulation of neurogenic and myogenic differentiation markers while changes in focal adhesion morphology were also observed. The topography-induced differentiation could be observed on different ECM compositions but more evident on selected ECM that directly induced differentiation. Our results indicated a direct effect of FAK activity on topography-induced gene expression. Meanwhile, in the neuronal differentiation of hESCs on nanogratings, the high actomyosin contractility induced by a nano-grating topography was crucial for neuronal maturation. Treatment of cells with the myosin II inhibitor (blebbistatin) and myosin light chain kinase inhibitor (ML-7) greatly reduces the expression level of microtubule-associated protein 2 (MAP2). Taken together, our findings may explain how hESCs and hMSCs can sense and transduce nanotopographical signals through focal adhesions and actomyosin cytoskeleton contractility to induce differential gene expression and to regulate stem cell differentiation.

Session No.: S16-06 Invited Speaker

Ligand Mobility Triggers Adhesion Transformation and Podosome Formation

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Matrix-activated integrins form different cell-matrix adhesion structures and there is a question whether traction forces can determine the type of adhesion. High traction forces play an important role in formation of focal adhesion but do physical factors affect other adhesions. . We have explored the role of force in podosome development using matrix ligands bound to fluid supported membranes that do not support traction forces. With fluid RGD-membranes, fibroblasts that normally do not form podosomes on rigid matrices will form podosomes within 45 minutes. Podosomes are defined by doughnut-shaped RGD rings and characteristic core components, including F-actin, cortactin, and Arp2/3. We employed RGD-membranes with nano-partitions and demonstrated that dense partitions (lines spaced by 1μm) in RGD-membranes suppressed podosome formation when contractions pulled integrin clusters to the lines and generated force on them. When cells were unable to generate forces on clusters (e.g. with wider separations of partitions (4μm) or myosin inhibition), cells formed podosomes. In addition, within a single cell, clusters over continuous RGD-membranes will form podosomes whereas clusters pulled to barriers will not. Inhibition of formin or actomyosin activity does not suppress podosome formation on RGD-membranes. However, inhibition of PI3K activity or activation of RhoA-mediated cellular contractility block podosomes. The local recruitment of phosphoinositide 3-kinase and sequential increase of phosphatidylinositol (3,4,5)-trisphosphate level plus recruitment of PTEN are associated with the podosome formation process. Thus, we suggest that force on integrin clusters will stimulate regular adhesion formation, whereas PI3K activation at an integrin cluster in the absence of force will stimulate podosome formation.

Session No.: S16-07 Invited Speaker

Functionalization of Hydrogels with Biomimetic Peptides to Emulate the Stem Cell Niche Microenvironment

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In recent years, human mesenchymal stem cells (hMSCs) have become increasingly popular as a cell source for tissue repair and regeneration. However, direct administrations of hMSCs into tissue defects often lead to limited regeneration due to significant cell loss and death as a result of the lack of mechanical and biochemical support from the surrounding environment. To develop effective carrier for the successfully delivery of hMSCs, increasing research emphasis has been placed on the "bio" part of biomaterials to emulate the microenvironment of the cells. Rather than being biologically inert, novel biomimetic biomaterials with intricate biological functions which can proactively interact with living cells and tissues by design have been presented by a number of recent studies. Insights gained from these studies also feedback to the optimization of biomaterial design leading to the development of novel biomimetic biomaterials with enhanced functions and efficacy. In our lab, we have shown that functionalization of the hydrogels with biomimetic peptides promotes the differentiation of the hMSCs. In addition to the biofunctionalization, the physical functions of the biomaterials are also critical to the interaction of the stem cells with the biomaterials. We have developed a series of supramolecular hydrogels with unique properties such as resilient mechanical property, fast relaxation, self-healing, bioadhesiveness, injectability, and promoting recruitment of endogenous cells. These hydrogel properties are not only desirable for potential clinical applications of these hydrogels but also useful for studying the effect of microenvironmental mechanical cues on stem cell behaviors.

Session No.: S17-01 Keynote Speaker

Functional Tissue Engineering for Annulus Fibrosus Regeneration

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Degenerative disc disease (DDD) is a leading cause of low back pain which has evolved into a serious global health problem and significantly contributes to healthcare costs. While it is promising to repair degenerated intervertebral discs (IVDs) by replacing them with biomimetic ones prepared using tissue engineering techniques, such an approach largely relies on the efficient construction of annulus fibrosus (AF), a critical load-bearing component of IVD. However, major challenges remain toward fabricating AF replacements that are biologically and functionally comparable to native AF tissue, mainly due to the tremendous complexity of AF tissue at cellular, biochemical, microstructural, and biomechanical aspects. This talk will provide an overview of current AF regeneration strategies. Following that, recent studies from our group focusing on the development of new scaffold materials and stem cells for AF tissue engineering will be introduced. In brief, we synthesized a series of biodegradable polyurethanes with similar elastic modulus as AF tissue and fabricated nanofibrous scaffolds using them. Meanwhile, we identified multipotent AF-derived stem cells (AFSCs) from AF tissues. Using these scaffolds and AFSCs, we found that the elastic modulus of scaffold material markedly affects the biochemical and biomechanical profiles of AFSCs and the matrix they produce. Further, we applied dynamic mechanical stimulation to AFSCs and found that their anabolic and catabolic metabolisms of matrix components were significantly dependent on the magnitude, frequency and duration of mechanical stimulation. Findings from these studies may provide new insights toward developing engineered AFs whose biological characteristics and mechanical functions are comparable to native AF tissues.

Session No.: S17-02 Invited Speaker

Osteochondral Tissue Engineering Based on Biomimetic Osteochondral Scaffold Contained Calcified Cartilage Layer Compounding with Adscs in Vitro and Vivo

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To investigate the effects of biomimetic osteochondral scaffold contained calcified cartilage layer (CCL),compounding with ADSCs on regeneration of the osteochondral defect in vivo. We fabricated a novel biomimetic osteochondral scaffold with CCL using slik fibroin (SF) and hydroxyapatite (HA) by the combination of paraffin-sphere leaching and modified temperature gradient-guided thermal-induced phase separation (TIPS) technique. ADSCs were seeded into the scaffold. The structure of scaffold, the cell adhesion, proliferation and viability on scaffold were observed by SEM, micro-CT, Dead/Live staining and other tests. The osteochondral defect model on rabbit bilateral knees were established, and implanted with the CCL+ADSCs, Non-CCL+ADSCs, CCL (no cells) and non-treated groups. At 4w, 8w and 12w after implantation, gross observation score, histological and immunohistochemical assessment, biochemical quantitative and biomechanical testing of new osteochondral tissue, micro-CT scans for new bone, were executed. The scaffold had a consecutively overlapping trilayer structure with different densities and pore structures, approximately bionic the normal osteochondral structure. SEM, Dead/Live staining and other tests had shown good ability of cell adhesion, proliferation on scaffold. The CCL effectively isolated the cells among chondral and osteogenic layers. The cartilage regeneration in CCL+ADSCs group was better than Non-CCL+ADSCs group and CCL group, mainly reflected in flatness and integrity of cartilage. The content of GAG and type II collagen in new cartilage tissue in CCL+ADSCs group more than the other groups. The results meant that the CCL contributed to the growth of cartilage. The biomimetic osteochondral scaffold contained CCL approximately bionics the normal osteochondral structure, the CCL can isolate the different microenvironment for the growth of cartilage and bone. The scaffold compounding with ADSCs satisfactory regenerate the rabbit osteochondral defect, the CCL can accelerate the growth of cartilage tissue, especially the secretion of ECM, and the ADSCs contributes to the quality and intensity of new bone.

Session No.: S17-03

Rabbit Xenotransplantation Model for Evaluating Human Chondrocyte Sheets for Articular Cartilage Repair at 12 Weeks

Takumi Takahashi[1], Masato Sato[1], Eriko Toyoda[1], Hideyuki Maruki[2], Daichi Takizawa[1], Ayako Tominaga[2], Eri Okada[1], Miki Maehara[1], Masahiko Watanabe[1]

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In 2014, we concluded a clinical study using autologous chondrocyte sheets, verifying their safety and effectiveness in treating osteoarthritis in the knee, and now, we are preparing for a Japanese Ministry approved clinical study using allogeneic chondrocyte sheets. We previously reported the usefulness of a rabbit full-thickness osteochondral defect model for evaluating human chondrocyte sheets at 4 weeks through xenotransplantation. In this study, we attempted to evaluate the regenerative effects of human sheets at 12 weeks. Under the approval and guidance of the Tokai University Ethics Committee, articular chondrocytes and synoviocytes were obtained from patients undergoing total knee arthroplasty (TKA). TKA sheets were created as per the first clinical study. Full-thickness osteochondral defects were created in knees of 16 female Japanese white rabbits: 6 defect group, 10 sheet transplantation group. For 4 weeks after transplantation, tacrolimus, an immunosuppressant, was administrated intramuscularly, and at 12 weeks, animals were sacrificed for histological evaluation. Safranin O sections were scored using a modified International Cartilage Repair Society (ICRS) scoring system. Linton incapacitance tester was used to evaluate post-surgical pain. In the sheet transplantation group, weight distribution ratios improved (Day 1: 34.0±0.5%, Day 28: 50.8±1.5%) but worsened (Day 42: 37.9%±4.1%) without recovering (Day 84: 44.1±2.3%). Compared to previous ICRS scores at 4 weeks (control 20.1±2.0, sheet 30.4±2.8), scores at 12 weeks (control 25.8±1.6, sheet 18.2±2.8) were significantly lower in the sheet group. In the sheet group, infiltration by immune cells was observed preventing repair of both the subchondral bone and articular cartilage, and very few human cells were detected. Significant complications from side-effects and the immunoprivileged nature of cartilage compelled us to stop immunosuppression after 4 weeks. As a result, transplanted human cells were rejected, and cartilage repair was significantly impeded. Our results indicated that continuous immunosuppression is essential in our xenotransplantation model.

Session No.: S17-04

N-Cadherin Mimetic Peptide Hydrogel Promotes Chondrogenesis of Human Mesenchymal Stem Cell via Suppression of Wnt/ β--Catenin Passway

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N-cadherin, a major transmembrane protein component of adherens junction mediate cell-cell interactions and intracellular signaling that are important to cell behaviors and organ development. Previous studies have identified mimetic peptides that possess similar bioactivity as that of the N-cadherin. Here, we incorporated an N-cadherin mimetic peptide sequence with the self-assembling KLD peptide, and the resultant peptide is capable of self-assembling in to fibrous hydrogels containing the pendant N-cadherin peptide. Encapsulation of human mesenchymal stem cell (hMSC) in these hydrogels showed enhanced deposition of cartilage specific extracellular matrix rich in proteoglycan and type II collagen compared to the control without the N-cadherin peptide after 14 days of chondrogenic culture. This finding suggests that the peptide hydrogels functionalized with the N-cadherin peptide effectively promoted the commitment of the hMSCs towards the chondrogenic lineage. We further investigated the underlying molecular mechanism of the enhanced chondrogenesis. Our western blot data showed lower expression level of -catenin in the N-cadherin peptide hydrogels compared to that in the control peptide hydrogels in early stage of chondrogenesis. Phosphorylation of the -catenin leads to its degradation and reduced activation of the osteogenic gene expression, and this may have contributed to the enhanced chondrogenesis of hMSCs in the N-cadherin peptide hydrogels. Taken together, the self-assembly N-cadherin mimetic peptide hydrogels serves as a tailorable chondrogenic platform for stem cell based cartilage regeneration.

Session No.: S17-05

Scaffolds with Radially Oriented Pores Facilitate in Situ Inductive Regeneration of Osteochondral Defects

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Abstract: It is hard to realize structural and functional regeneration of critical osteochondral defects spontaneously by inherent self-healing ability without artificial interference. Cartilage regeneration based on traditional tissue engineering has been impeded on the path from laboratory bench to bedside. Evoking and supporting the self-healing and self-remolding process of cartilage regeneration simply by introducing a cartilage inductive scaffold would be a promising way to push forward the clinical application.[1] A proper biomaterial-based scaffold plays an essential role in this process. Scaffolds with cell-penetration facilitating structure and chondrogenesis inductive bioactivity would promote the in situ inductive regeneration of osteochondral defects. Radially oriented pores could be easily granted to scaffolds based on poly(lactic-co-glycolic acid) or hyaluronic acid through thermally induced phase separation.[2] In vitro evaluation proved less special obstacle for cell penetration, while in vivo implantation confirmed more obvious simultaneously regenerated cartilage and subchondral bone in poly(lactic-co-glycolic acid) scaffolds with oriented rather than random porous structure, even though cartilage specific extracellular matrices deposition is not as high as native cartilage. Bioactive hyaluronic acid could function as a chondrogenesis inductor, leading to chondrogenetic of bone marrow stem cells as well as increase of cartilage specific extracellular matrices deposition.[3] In vitro culture of bone marrow stem cells within scaffolds based on hyaluronic acid proved spontaneously cells aggregation. Radially oriented pores and bioactivity of hyaluronic acid lead to improved full regeneration of cartilage and subchondral bone with great integration and higher deposition of matrices. Keywords: cartilage regeneration; in situ inductivity; oriented pores; poly(lactic-co-glycolic acid); hyaluronic acid. Acknowledgements This study is financially supported by the Natural Science Foundation of China (21434006, 21374097). References [1] Vanden Berg-Foels WS, Tissue Engineering Part B: Reviews. 2013;20:28-39. [2] Zhang T, et al., Journal of Biomaterials and Tissue Engineering. 2014;4:1030-9. [3] Kawasaki K, et al., Journal of cellular physiology. 1999;179:142-8.

Session No.: S18-01 Keynote Speaker

Hybrid Nanoconstructs for Biomedical Photonics in the Second Biological Window

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Optical loss in biological tissue is determined by scattering and absorption. The stronger scattering is, the shorter the wavelength is. The scattering causes not only simple loss but also causes blur of images. Because of this reason, florescence biomedical imaging has been moved to be in longer wavelength for decades. If the wavelength is too long, tailing of the infrared absorption by molecular vibration prevents the propagation of light. The optical loss spectrum by the scattering and absorption forms a valley in near infrared (NIR) wavelength range called “biological window.” The first biological window (FBW), 800-1000 nm, has been used long time after the FDA approval of indocyanine green (ICG) in 1959, which can be detected by conventionally used silicon imaging devices. Recently, the wavelength over-1000-nm (OTN) has been attracting interests for fluorescence biomedical imaging since one can expect ten times deeper observation depth (several cm) in the OTN-NIR range in comparison with the imaging in the FBW. In the OTN-NIR, people starts calling 1000-1450 nm one to be the second biological window (SBW or NIR II) and 1500-1800 nm one to be the third biological window (TBW or NIR III). The imaging in the OTN-NIR range was enabled by the commercialization of InGaAs CCD camera, which can visualize the light in the NIR II/III. Since 2005, the authors have developed the florescent materials and imaging systems for the OTN-NIR range. In this presentation, we will review the design and processing of nanoconstructs for the fluorescence bioimaging, nanothermometry and photodynamic therapy in the OTN-NIR (NIR II/III) wavelength region.

Session No.: S18-02 Keynote Speaker

Mechanical Properties of SF/PCL Patches Modulate the Formations of hBMSC Microtissues/Patch and Influence Cardiomyogenesis of hBMSC

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Since high similarity between in-vitro microtissues of cells and in-vivo normal tissues, the strategy to fabricate 3D microtissues has been widely investigated for tissue engineering. Moreover, microtissues would enhance the secretion of cytokines and differentiations of BMSC to specific tissues in-vitro compared to those of 2D cell monolayers have been documented. Here, cardiac patches composed of poly (ε-caprolactone) (P) grafted by silk fibroin (SF) of varying β-sheet contents (or crystallinity, %) were produced to regulate the mechanical property of substrates (PS) for investigating the effects of the property on the proliferation and cardiomyogenesis of hBMSC in-vitro. The crystallinities of SF were examined by ATR-FTIR spectra and analyzed by Fourier-deconvolution method. Young\'s modulus, AFM and SEM were employed to characterize mechanical property and surface topography of the patches. Different preparing techniques such as ethanol annealing were employed to induce the crystallinities of SF with varying from 20% to 44% (e.g., PS20 and PS44, respectively). The values of Young\'s modulus of PS patches were increased with increasing crystallinity of SF. In addition, the topological images of SEM and AFM shows that different degrees of nanoscale of SF particles were distributed on the surfaces of the patches especially in PS44 one. After three days of cultivation, hBMSC were well proliferated on tested patches. Interestingly, microtissues of hBMSC onto PS20 and PS30 patches were observed while 2D hBMSC monolayers were found on PS37 and 44 patches, respectively. Notably, cardiac specific proteins such as connexin 43 of hBMSC microtissues/PS hybrid patches induced by 5-aza showed significantly higher expressions than those of hBMSC monolayer/PS ones ($p<0.05$, $n=3$). In conclusion, 3D hBMSC microtissues/PS hybrid cardiac patches were produced by regulating Young\'s modulus of PS patches via varying crystallinity of SF that promotes cardiomyogenesis of hBMSC.

Session No.: S18-03 Invited Speaker

Preparation of a Plga-collagen Hybrid Scaffold with Controlled Mechanical Properties and Pore Structures

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Mechanically strong and highly porous scaffolds are desirable to promote cell adhesion, proliferation, differentiation, and guide new tissue formation. Porous scaffolds from synthetic polymers such as poly(lactic-co-glycolic acid) (PLGA) have a broad range of mechanical properties but lack cell affinity. By contrast, porous scaffolds from naturally derived polymers such as collagen have advantages of good cell adhesion and adequate hydrophilicity, but they are mechanically weak. To overcome the problem, we have developed various types of hybrid scaffolds by combining their respective advantages of PLGA and collagen. In the present study, we further designed and prepared a hybrid scaffold with sandwich-like structure of a PLGA mesh in the middle and two highly-ordered porous collagen sheets. To prepare the hybrid scaffold, type I collagen solution and sieved ice particulates were mixed and then poured into a plastic mold with a PLGA mesh placed in the middle of the mixture. The whole construct was freeze-dried to form porous structure and then cross-linked by carbodiimide chemistry. SEM observation revealed the hybrid scaffold had large pores with controlled diameters and had interconnected small pores. The in vitro cell culture of bovine chondrocytes showed that the controlled pore structures facilitated homogeneous cell distribution and cartilage tissue formation. The sandwich-like PLGA/collagen hybrid scaffold with controlled mechanical properties and pore structures will be beneficial not only in effective cartilage regeneration but in surgical handling.

Session No.: S18-04 Invited Speaker

Osteoanabolic Implant Materials for Orthopaedic Treatment

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Osteoporosis is becoming more prevalent due to the aging demographics of many populations. Osteoporotic bone is more prone to fracture than normal bone, and current orthopaedic implant materials are not ideal for the osteoporotic cases. A newly developed strontium phosphate (SrPO4) coating is reported herein, as applied to Ti-29Nb-13Ta-4.6Zr (wt.%), TNTZ, an implant material with a comparative Young's modulus to that of natural bone. The SrPO4 coating is anticipated to modulate the activity of osteoblast and osteoclast cells, in order to promote bone formation. TNTZ, a material with excellent biocompatibility and high bio-inertness was pretreated in a concentrated alkaline solution under hydrothermal conditions, followed by a hydrothermal coating growth process to achieve complete SrPO4 surface coverage with high bonding strength. Owing to the release of Sr ions from the SrPO4 coating and its unique surface topography, osteoblast cells demonstrated increased proliferation and differentiation, whilst the cellular responses of osteoclasts were suppressed, compared to the control case, i.e. bare TNTZ. This TNTZ implant with a near physiologic Young's modulus and a functional SrPO4 coating provides a new direction in the design and manufacture of implantable devices used in the management of orthopaedic conditions in the osteoporotic individuals.

Session No.: S18-05

Well-controlled 3D Surface Modification of Electrospun Silk Nanofibers with Zno by Atomic Layer Deposition for Enhanced Photocatalytic Activity

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Metal oxides such as ZnO have attracted great attention attributed to their interesting reactivity and widely applications in various fields. The combination of these unique properties of metal oxides with organic materials has demonstrated to be feasible to develop novel functional biomaterials for biomedical applications. Particularly, the application of nanostructured biomaterials enables a one-step creation of nanostructure of metal oxides through deposition with great ease and well-controlled morphology. In this study, we explored the feasibility of conformably 3D coating ZnO for silk electrospun nanofibrous materials using atomic layer deposition (ALD) to develop silk/ALD composite materials and studied the photocatalytic activities. The effects of process temperature on the ZnO layer growth efficiency such as morphology, thickness, crystallinity were investigated, and the optimal temperature was obtained. The results from SEM, TEM, XRD, and EDS indicated that the ZnO layer deposited on the electrospun silk nanofibers was conformal, the integrity of the silk nanofibers were maintained during the ALD process, and the crystal structure of ZnO layer obtained at temperatures higher than 100 oC was hexagonal wurtzite structure. In addition, the ZnO/silk materials remained high flexibility and elastic mechanical properties. The photocatalytical activity of ZnO was further confirmed with Rh-B color fading upon UV irradiation using ZnO nanotubes obtained by calcining the silk nanofibers. Therefore, ALD has demonstrated as a desirable approach for the surface modification of silk biomaterials with metal oxide with additional functionalities. This provides a general platform for developing organic/metal oxide composite materials with enhanced activity.

Session No.: S18-06

Comparing Hybrid Nano-microfibrous Constructs of Plastic Compressed Collagen - Electrospun Plga: Collagen Content Percentage as Variable

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Collagen is a natural polymer with extensive application in tissue engineering and regenerative medicine. Collagen hydrogel, as a common scaffold for rapid introduction of cells, suffers from low mechanical strength. Plastic compression of collagen hydrogel was introduced as a method to improve its mechanical properties. Such a scaffold, is however still weak and has no dimensional stability. As a result, researchers have performed the procedure of plastic compression over a supporting substrate so that a hybrid construct including collagen could be achieved. Our studies have shown that the procedure of collagen hydrogel compression (with the conventional hydrogel preparation formula), leads to a very dense nanofibrous construct with limited chance for cells to infiltrate. In this study, we have fabricated hybrid nano-micro, natural-synthetic scaffolds of compressed collagen-electrospun PLGA. Collagen hydrogels with different amounts of collagen (80 percent to 20 percent) were compressed over an electrospun sheet of PLGA. The scaffolds were then seeded by 3T3 fibroblast cells, and were incubated for 1, 4, and 7 days in vitro for electron microscopy, metabolic activity and staining studies. SEM imaging of non-seeded scaffolds showed that decreasing the share of collagen in hydrogel formula leads to fibrous constructs with lower fibrous densities. SEM imaging of cell-seeded scaffolds revealed that all the scaffolds support cell adhesion and proliferation. According to MTS assay, the highest metabolic activity was seen in the scaffold with lowest collagen. A similar trend was observed in the study of depth of cell infiltration using confocal microscopy of DAPI- Phalloidin stained cells. Based on this study, we conclude that decreasing the collagen content in the formula of collagen hydrogel for plastic compression, not only attracts more attention to this expensive biopolymer due to decreasing the cost, but also leads to improved cell-scaffold interaction and higher metabolic activity of cells.

Session No.: S18-07

Effects of Addition of Acrylic Compounds in Apatite/Collagen Porous Composites with Gamma-Ray Irradiation on Mechanical and Biodegradable Properties

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The control of material properties, such as mechanical and biodegradable properties, considering sterilization methods for inorganic and organic composites is of great importance for developing scaffolds for tissue engineering and biomedical devices. Although apatite/collagen porous composites are a promising artificial bone and scaffolds, the mechanical and biodegradable properties have been generally controlled by a chemical crosslinking such as glutaraldehyde, water soluble carbodiimide, or some enzymes which shows often cytotoxicity. In this study, the effects of addition of acrylic compounds, polyethyleneglycol-diacrylate (PEGDA) in apatite/collagen porous composites with gamma-ray irradiation were investigated. The apatite nanocrystals were mixed into the collagen solutions at pH6.8 including D-phosphate buffer saline (D-PBS), which was freeze-dried. The weight ratio of apatite and collagen was fixed at 3:1. The composite was mixed with D-PBS including the fixed amounts of PEGDA of which composites were irradiated by the gamma-ray at 25 kGy in wet condition, and again freeze-dried. The molecular weights of PEGDA and dose rate of gamma-ray were changed; the swelling ratio of the composites were increased and the young's modulus of the composites with 80% porosity were apparently decreased with the molecular weights and the quantity of PEGDA. Interestingly, the dose rate of gamma-ray increased the compressive strength at 100 times compared with the composite with thermal cross-linking method. The degradability of the composites was evaluated with collagenase at 10 units/mL by the change of weight. The molecular weight and quantity of PEGDA apparently shows the decrease of degradability. After 4 week's tests, the degradability of the composites without PEGDA was almost 70%; however the composites with PEGDA were inhibited at almost half degradability. In conclusion, the addition of PEGDA in apatite/collagen porous composites was effective for the control of mechanical and degradable properties.

Session No.: S19-01 Keynote Speaker

How to Prevent Arrhythmogenesis During Cardiac Cell Therapy?

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One major obstacle in cardiac cell therapy is that cells derived from embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are generally immature and tend to display the structural and functional attributes of fetal cells, rather than the adult phenotype, thus increasing the risk of arrhythmogenesis following delivery. We report a method for improving features of maturation in murine and human embryonic stem cell-derived cardiomyocytes (m/hES-CMs). We have found that coculturing m/hES-CMs with endothelial cells improves their maturity and upregulates several microRNAs. Delivering four of these microRNAs, miR-125b-5p, miR-199a-5p, miR-221, and miR-222 (miR-combo) to m/hES-CMs resulted in improved sarcomere alignment and calcium handling, a more negative resting membrane potential, and increased expression of cardiomyocyte maturation markers. Although this could not fully phenocopy all adult cardiomyocyte characteristics, these effects persisted for two months following delivery of miR-combo. Luciferase assay demonstrated that all four miRNAs target ErbB4, and siRNA knockdown of ErbB4 partially recapitulated the effects of miR-combo. In summary, a combination of miRNAs induced via endothelial coculture improves ES-CM maturity, in part through suppression of ErbB4 signaling.

Session No.: S19-02 Keynote Speaker

Injectable Biomaterials for Cardiac Tissue Engineering

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Biomaterials have significant potential to treat various cardiac conditions. In particular, new therapies for myocardial infarction are needed given that heart failure post-myocardial infarction continues to a leading cause of death. Biomaterial and tissue engineering approaches to myocardial repair are providing exciting new possibilities. Injectable biomaterials are particularly attractive since they have the potential to be delivered via a minimally invasive, catheter-based approach, thereby requiring less recovery time and reducing the chances of infection. Recent developments and translational progress with injectable biomaterials designed specifically for treating the heart will be discussed.

Session No.: S19-03 Keynote Speaker

Fabrication of Poly (Ethylene Glycol) Hydrogel Composites as Heart Valve Substitutes

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The number of patients requiring heart valve replacement increasing from approximately 290,000 in 2003 to over 850,000 in 2050. Current devices for valve replacement have significant limitations. For example, mechanical valves require anticoagulation in the lifetime of the patients. Bioprosthetic heart valves have relatively poor durability due to leaflet mineralization and mechanical fatigue. In our study, poly(ethylene glycol) (PEG) hydrogel composites with mechanical property similar to native valves were fabricated as artificial heart valve leaflets, which likely provide advanced solutions to the limitations of current treatments. Anisotropic mechanical properties were established in PEG hydrogels by crosslinking stripes of different molecular weight PEG diacrylate (PEGDA) using a photolithographic patterning method. Biomimetic PEG–peptide hydrogels by tethering the cell-adhesive peptide RGDS and incorporating a collagenase-degradable peptide into the polymer network were prepared for heart valve tissue engineering. Furthermore, laminated of PEG-protein fiber composites were fabricated by layer-by-layer assembly of PEG hydrogels and eggshell membranes (ESMs), which had an average elastic modulus and elongation rate comparable to those from native aortic valve leaflets. ESMs showed fast shape recovery in aqueous environment, likely due to the improved flexibility of macromolecular chains by water lubrication. Thus, ESMs can retain shape memory characteristic in PEG-protein fiber composites considering the large volume of water (~80%) in PEG hydrogels. The ESM layers are the major stress-bearing components during tension and provide strength to retain original shapes during diastole, while the PEG hydrogel layers absorb energy during compression. The PEG-protein fiber composites also showed good resistance of enzyme degradation and no immune rejection in vivo, and the outer layers of PEG hydrogels prevented the calcification of inner protein fibers. Thus, this study lays the basis for fabrication of PEG composites as artificial valve leaflets following valve-inspired design principles.

Session No.: S19-04 Invited Speaker

Create a Biological Pacemaker

Chuansen Zhang[1]

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Despite the utility of electronic pacemakers in treating heart block and/or sinus node dysfunction, there is need of alternatives that more completely reproduce normal function. In recent years, a number of studies have been published about the therapy of the diseases with a biology-based method. These studies have focused on the use of cell transplant and gene transfer for the therapy. However, both gene transfer and cellular transplantation are far from completely reproducing normal function of native cardiac conduction system. The above-mentioned methods only provided a palliation of heart block and/or sinus node dysfunction. In particular, transgenic expression is transient and cells do not stay in the desired injection site. So the transplantation of a tissue engineering cardiac pacemaker (TECP) or conduction tract (TECT) may be ideal in treatment of heart block and/or sinus node dysfunction. In our laboratory, the studies of biological pacemakers have been completed as following. First, the optimized source of seeding cells in biological pacemaker was obtained. We have isolated cardiac progenitor cells derived from embryonic heart tubes, brown adipose-derived stem cells derived from interscapular brown adipose tissue and very small embryonic-like stem cells derived from marrow. Second, tissue engineering biological pacemakers have been created. TECP were fabricated by seeding the CPCs-derived pacemaking cells into Matrigel. The transplanting of TECP into hearts can establish a new pacemaker which has the characteristic similar to original sinus node. TECT were created by seeding the CPCs-derived conduction cells into gelatin foam in vitro. The transplanting of TECT into hearts can function as an electrical conduit and, ultimately, may offer a substitute treatment to conventional pacing therapy.

Session No.: S19-05 Invited Speaker

Cardiac Progenitor Cell Sheet for Heart Tissue Repair

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Recently cell-based therapies have emerged as an alternative for cardiac transplantation and a method for repair of damaged heart. Among the tested cell types progenitor cells isolated from postnatal heart are one of the most potentially applicable for cell-based therapy due to their considerable regenerative potential for impaired heart tissue shown in experimental and clinical settings. However, poor retention and viability of transplanted cells delivered by direct injection of cell suspension into impaired myocardium or into coronary circulation are the main obstacles in this field restricting therapeutic benefits of cell therapy. To overcome these challenges cell sheet (CS) technology has been developed as a means of permitting longer retention and better viability of graft cells. We assembled c-kit+ Lin- cardiac progenitor cells and cardiosphere-derived cells into scaffold-free CSs using temperature-responsive cell culture dishes. Both type of CSs were shown to secrete growth factors and extracellular matrix (ECM) proteins providing an appropriate cellular micro-environment. Implantation of these constructs on epicardial surface in a rat model of acute myocardial infarction provided better cell survival and engraftment than control delivery of the same amount of cells by injection. Intensive migration of CSC from the sheets to area of infarction was observed. Transplanted cells proliferated within CSs and expressed markers of endothelial and cardiogenic differentiation. Significant vascularization of CSs and underlying border and infarct zones were observed. CSs implantation reduced post-infarction structural myocardial remodelling and fibrosis. It is intriguing to note that CS implantation stimulated cardiomyocytes proliferation in both – border and infarction zones. Our results suggest that delivery of cardiac progenitor cells as cell sheets to infarcted heart could be a promising approach to stimulate myocardial angiogenesis and induce regeneration. Possible mechanisms of angiogenic and regenerative effects of cardiac progenitor cell sheets implantation will be discussed.

Session No.: S19-06

Improve Cardiac Repair with Natural or Artificial Scaffold with Natural Component

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Cardiac tissue engineering using biomaterials with or without a combination of stem cell therapy offers a new therapeutic option for repairing infarcted heart. we found that natural scaffold --human heart valve-derived scaffold (hHVS) may provide a clinically relevant novel biomaterial for cardiac repair. Upon anchoring onto the hHVS, post-infarct murine bone-marrow(BM) c-kit+ cells exhibited an increased capacity for proliferation and cardiomyogenic differentiation in vitro. When used to patch infarcted heart in a murine model of myocardial infarction, either implantation of the hHVS alone or c-kit+ cell-seeded hHVS significantly improved cardiac function, and reduced infarct size; while c-kit+ cell-seeded hHVS was even superior to the hHVS alone. on the basis of these findings, we hypothesized cardiac nature protein (NP), especially elastin and collagen, in hybrid polycaprolactone (PCL) electrospun nanofibrous scaffold could be effective as cardiac-mimicking patch. After optimal ratio of elastin and collagen with PCL in electrospun sheets (80% NP/PCL) was selected based on cytocompatibility and mechanical characteristics, BM c-kit+ cells were anchored onto NP/PCL scaffold, which also exhibited increased proliferative capacity compared with those seeded on PCL in vitro. Moreover, we examined the improvement of cardiac function in MI mice by cell-seeded cardiac patch. Both 80% NP/PCL and c-kit+-seeded 80% NP/PCL effectively improved cardiac function after 4 weeks of transplantation, with reduced infarction area and restricted LV remodeling. C-kit+-seeded scaffold was superior to NP/PCL alone and both superior to PCL. Our in vitro and in vivo observations provide some new choices for myocardial infarction treatment both through hHVS-based and cardiac nature protein (NP)/PCL electrospun nanofibrous-based biomaterials.

Session No.: S20-01 Keynote Speaker

Environmental Reprogramming of Fibroblasts for Hair Follicle Neogenesis

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Organ development is a complicated yet well-orchestrated process of self-organization involving different cell types. Despite the growing knowledge of the mechanisms for organ development, little is known about how they can be re-elicited in adults for regeneration. We found that cell-free extract from the embryonic skin of perifolliculogenetic stage conferred adult fibroblasts with the competence to form new hair follicles. IGF and Wnt signaling were activated and required for this process in adult fibroblasts. Transcriptome and functional analysis showed that fibroblasts were brought to a state with hair inductive property after exposure to embryonic cell-free extract. The gained hair inductive competency was reversible and lost after a washout period. Through proteomics analysis, we identified three secreted proteins enriched in the embryonic skin that together were essential and sufficient to induce new hair follicles in adult mice. Therefore, extracellular proteins can confer the competency for regeneration on fibroblasts by partially reprogramming them toward embryo-like state in which they can re-engage in developmental interactions. Identification of factors to recreate tissue-specific embryonic extracellular context can be a novel strategy to promote regeneration in various adult organs.

Session No.: S20-02 Invited Speaker

Origin of Functional Niche and Long Term Hair Follicle Stem Cells

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Long-term adult stem cells sustain tissue regeneration throughout the lifetime of an organism. They were hypothesized to originate from embryonic progenitor cells that acquire long-term self-renewal ability and multipotency at the end of organogenesis. The process through which this is achieved often remains unclear. Here, we discovered that long-term hair follicle stem cells arise from embryonic progenitor cells occupying a niche location that is defined by attenuated Wnt/b-catenin signaling. Hair follicle initiation is marked by placode formation, which depends on the activation of Wnt/b-catenin signaling. Soon afterwards, a region with attenuated Wnt/b-catenin signaling emerges in the upper follicle. Embryonic progenitor cells residing in this region gain expression of adult stem cell markers and become definitive long-term hair follicle stem cells at the end of organogenesis. Attenuation of Wnt/b-catenin signaling is a prerequisite for hair follicle stem cell specification because it suppresses Sox9, which is required for stem cell formation.

Session No.: S20-03 Invited Speaker

Nanofibrous Scaffolds Combining Platelet-Rich Plasma and Adipose-derived Stem Cells for Skin Regeneration

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Composite nanofibrous scaffolds made from biocompatible biomaterials and platelet-rich plasma (PRP) were prepared as nano-structured membranes for skin regeneration by co-delivery of adipose-derived stem cells (ADSCs) and platelets (growth factors). ADSCs could promote wound healing when they differentiate into endothelial cells and increase physiologically significant levels of angiogenic cytokines at wound sites, which can up-regulate angiogenesis and vasculogenesis. PRP, a concentrated source of autologous platelets, contains (and releases through degranulation) several different growth factors and other cytokines that could stimulate healing of soft tissue. PRP was successfully electrospun into nanofibers with hyaluronic acid, chitosan, and gelatin with average fiber diameters in the range of 126 to 248 nm. The nanofibrous membranes have good tensile strain, good water sorption ability, and water vapor transmission rates similar to wet human skin. The nanofibrous membranes show time-dependent slow release of growth factors (EGF, PDGF and TGF-beta-1) and are suitable for attachment, growth, and differentiation of ADSCs in vitro. From animal studies, the nanofibrous membranes can attract fibroblasts and endothelial cells to the derma layer and the wound closure rate is better than gauze and commercial wound dressings. Histological stains indicated the rate of epithelialization was increased; the dermis became well organized to result in scarless wound healing with enhanced synthesis of collagen III and reduced synthesis of collagen I. ADSCs could migrate into wound sites and participate in forming new skin tissue.

Session No.: S20-04 Invited Speaker

Bioglass Promotes Wound Healing Through Affecting Behaviors of Repairing Cells

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Bioglass (BG) has shown application potential in wound healing due to its excellent antibacterial effects. Here, we proposed that the BG may accelerate wound healing through affecting behaviors of repairing cells involved in skin regeneration. After we investigated the effects of BG ion extracts on behaviors of human umbilical vein endothelial cells (HUVECs) and human dermal fibroblasts (HDF) in vitro, we found that BG ionic products with certain concentrations stimulated migration of HUVECs and HDFs, collagen expression and myofibroblast differentiation of HDFs and expressions of vascular endothelial growth factor, basic fibroblast growth factor as well as their receptors from both of cells. In addition, BG ionic extracts prevented death of HUVECs following hypoxia through blocking hemichannel opening while gap junctional communications and vascular endothelial cadherin in HUVECs treated with certain BG ion extracts were enhanced, indicating the stimulatory effects of BG on communications between HUVECs. Application of BG directly on full thickness wound of rat accelerated wound closure through stimulating vascularization and reducing inflammation response in wound site. Therefore, BG ionic products had strong effects on behaviors of ECs and fibroblasts, which could finally promote wound healing, indicating that BG has great application potential in wound healing.

Session No.: S20-05 Invited Speaker

Developing Keratin-alginate Sponges for Skin Regeneration

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Human-hair keratins have emerged as potential biomaterials of human origin. Nonetheless, there are currently limited clinical applications of hair keratins. In our recent study, the feasibility of producing a keratin-alginate composite sponge was explored, along with preliminary demonstration of its potential for skin regeneration applications. Keratin was extracted from discarded human hair samples using established methods. This was crosslinked with alginate using 1-Ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) to induce amide linkages between keratin and alginate. The resulting mixtures were cast and freeze-dried to produce 3D porous sponges. Fourier Transformed Infrared Spectroscopy (FTIR) and biochemical assays confirmed successful crosslinking of up to 82.1 ± 1.3 %. Tensile and compressive moduli were measured to be 219 ± 52.4 kPa and 191 ± 32.9 kPa, respectively. Notably, the crosslinked keratin-alginate sponges retarded water vapor transmission to the same extent as commercially available absorbent wound dressings. Sponges with higher proportions of keratin also showed lower water uptake capacities in comparison with sponges with higher alginate proportions. The composite sponges were able to support the attachment and proliferation of L929 mouse fibroblasts, suggesting a bioactive role that keratin played, possibly through the moderation of LDV cell adhesion motifs. Subcutaneous implantation of the crosslinked composite sponges into C57BL/6NTac mice over a period of 4 weeks showed minimal host tissue reaction and insignificant fibrotic capsule formation. More importantly, the composite sponges were able to support cellular infiltration, neo-tissue formation and vascularization more effectively than commercial collagen templates. In summary, our study demonstrated the feasibility of producing crosslinked keratin-alginate composite sponges with tuneable physical and mechanical properties, in vitro cell compatibility and in vivo biocompatibility. These results suggest that these composite sponges have the potential to be adapted for skin regeneration applications in the form of wound dressings or dermal equivalents.

Session No.: S20-06

Materials for Skin in Situ Regeneration: from Structure to Function

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Skin loss caused by trauma, burn and chronic diseases has become one of the most serious problems in clinic. Recent advances in regenerative medicine have developed diverse methods for skin repair and regeneration. However, there are still many obstacles, such as angiogenesis, hypertrophic scar, and loss of appendages, should be overcome to realize the full regeneration as the living skin, from structure to function. As a general principle of tissue engineering, the scaffold, acting as the artificial extracellular matrix (ECM), should present well-designed multiple chemical, physical and biological cues to build a suitable cellular microenvironment to achieve proper tissue function and regeneration. Compared to the instability and high cost of cell growth factors, the gene-activated materials (GAM) which can locally transfect cells and produce the required biosignals at the wound site, have attracted much attention. In this study, the gene-activated scaffolds were fabricated by incorporating pDNA-VEGF and siRNA-TGF-β1 with the collagen-chitosan scaffold, respectively. By in vitro and in vivo tests, the properties of enhanced angiogenesis and inhibited scar of the gene-activated scaffolds were evaluated. Furthermore, by the combination of gene technologies and stem cells therapy, the possibility of the regeneration of skin appendages such as hair follicle and sweat gland by the gene-activated scaffolds was also investigated.

Session No.: S20-07

Exosomes Collected from Human Induced Pluripotent Stem Cells Enhance Diabetic Skin Wound Healing

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Human induced pluripotent stem cells (hiPSCs) are regarded as a promising cell source for regenerative medicine. However, mainstream clinical use has been limited due to teratoma formation. Recently, stem cell-conditioned medium has attracted considerable attention as an effective tool for some disease models in regard to non-cell therapy. Researchers suggested that its mechanism is related to exosomes including microRNA and proteins. To date, a few reports have demonstrated exosomes derived from hiPSCs (hiPS-Exo). In this study, we investigated hiPS-Exo characterization, and also the potency of promoting diabetic wound healing by the application of hiPS-Exo. METHODS: In accordance with Yamanaka's report, we cultured hiPSCs (201B7, Riken) and collected hiPSCs conditioned medium. Exosomes derived from iPSCs culture medium (M-Exo), and hiPSCs conditioned medium (hiPS-Exo) were isolated using Magcapture (Wako). We did morphometric and flow cytometry analysis of M-Exo and iPS-Exo. We also evaluated the in vitro effects of hiPSC-Exo on both the proliferation and migration of diabetic mouse dermal fibroblasts by cell-counting and scratch assays. PBS, M-Exo, and iPS-Exo were respectively injected subcutaneously around a skin defect in a diabetic mouse model, and their effects on wound healing were assessed. RESULTS: Small vesicles (average diameter 120.0±24.5nm) were observed using transmission electron microscope only in the hiPS-Exo group. CD9, 63 and 81 were positive in hiPS-Exo group, and HLA-ABC was negative in both groups. hiPS-Exos stimulated the migration of diabetic mouse dermal fibroblasts in vitro, but did not stimulate proliferation. Injected hiPS-Exo to wound sites resulted in accelerated wound closure. CONCLUSIONS: Our findings suggest that hiPS-Exo enhances diabetic skin wound healing by accelerating fibroblast migration. hiPS-Exo might become a therapeutic option for diabetic ulcer. Further study in the future is required to fully understand its mechanism.

Session No.: S21-01 Keynote Speaker

Materiomic Screening of Topographical Cues That Bias Migration and Differentiation of Liver Progenitor Cells

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Materiomics has emerged as a powerful approach to screen for microenvironmental cues that influence stem cell differentiation decisions. Many developmental processes involve not only stem cell differentiation but also cell migration in synergistic way that determine organ or tissue morphology and functions. We have developed a chip on which cells can migrate on 144 runways per chip with different permutations of topographical cues that can differentially impact both cell migration and differentiation. We have used HepaRG which is capable of differentiation into hepatocytes or cholangiocytes in defined differentiation cell-culture medium that usually yields 60% hepatocytes and 40% cholangiocytes, to screen for topographies that preferentially supporting or preventing cell migration and biased differentiation into hepatocytes or cholangiocytes. Using surface profiler and high speed confocal imaging, we have correlated topographies that form clusters of cells with different ratios of hepatocytes and cholangiocytes. Cells in different clusters were further characterised for hepatocyte- or cholangiocyte-specific markers such as Albumin, CYP3A4, and CK19, as well as F-actin, E-Cadherin, beta-catenin and integrins. Using statistical classifiers based on molecular distributions of these markers, we can establish a clustering index with weighted combinations of all the relevant image features, and use the index to rank different degrees of biased cell migration and differentiation in the context of different topographical cues. This work will establish the basis of correlating topographies to both cell migration and differentiation decisions; and kick start a line of further mechanistic studies to unravel the mystery of how environmental cues impact the two processes that are often coupled in stem or progenitor cells.

Session No.: S21-02 Invited Speaker

Using 3D Printing and Biomems to Induce Neurophere

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Neuroma and neuron scar usually follow nerve injury and hinder nerve regeneration. Recently, stem cell therapy has been proved to be a potential treatment for nerve repair. In our lab, we are able to isolate adipose derived stem cells (ASC) and seed the cells on chitosan-coated surfaces to form spheres which causing the cells undergo transdifferentiation into neural linage cells (NLCs). However, it remains challenging to form spheres with single size which then makes it impossible to observe the optimized condition to transdifferentiate ASCs. The study aims to fabricate biochips that are able to form single size spheres and then evaluate the characteristic of neural spheres formed in various condition. We shaped polydimethylsiloxane (PDMS) biochips with different geometries, heights and diameters by 3D printer and bioMEMs technology and coated with chitosan. ASCs were seeded in the wells to form neurosphere. To aware the optimized condition to transdifferentiate ASCs into NLCs, light microscope was used to observe the regulation of well geometry, depth and diameter in sphere size. In addition, RT-PCR was applied to evaluate expression of neural marker for spheres in different condition. Images took by light microscope indicated the possibility of forming spheres with single size, and the sphere size could be regulated by the well condition. Besides, the PCR data revealed that certain size of neural spheres expressed relative high level of neural markers. In summary, we were able to tell the optimized condition of biochip to form neural spheres and produced great number of the unite size spheres which may be applied to improve nerve regeneration.

Session No.: S21-03 Invited Speaker

Engineering Cell Microenvironment Using Novel Hydrogels for Biomedical Applications

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With advances in micro- and nanoscale technologies, it has been possible to manipulate cells in microscale volumes with precision comparable to the natural systems to address challenges in medicine. In this talk, I will present the unique opportunity of microengineering in solving significant clinical problems in medicine, especially 3D microscale tissue engineering. I will begin by introducing engineering of cell microenvironment to generate 3D tissue constructs by assembling micro-engineered tissue blocks (mesoscale cell-laden hydrogels) using several approaches including magnetic force, acoustic power and electrostatic interaction. The tissue assembly process recapitulates the native tissue formation from repeating functional units (i.e., myofiber, lobule or nephron). Several bottom-up approaches (e.g., cell printing, microgel assembly) are demonstrated for engineering complex 3D tissue constructs with delicate microarchitecture, cell-cell interactions and internal vascular-like network that have not been achieved by conventional ‘top-down’ tissue engineering approaches. Examples of applying these approaches in tissue engineering, biomechanics, cancer biology, stem cell niche, cell-based biosensor, and high throughput drug screening will also be presented. The approaches for manipulating cells in microscale volume presented here hold great promise for developing complex tissue substitutes and cell-based systems for bio-sensing and drug screening.

Session No.: S21-04 Invited Speaker

Cell Responses to Nano-grooved Surfaces

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Anchorage-dependent mammalian cells are attached to the extracellular matrix (ECM), whose biochemical and physical cues dictate the fates of the cells. A substratum with a micro- or nanometre-scale 3-dimensional feature is a powerful tool for investigating cell responses to the ECM topographic structure. We fabricated a series of nano-grooved substrates containing grooves/ridges of 90 – 900 nm wide and 50 – 500 nm deep in order to investigate cell responses to nano-grooved surfaces. A general cell response to such anisotropic topography is cellular alignment and elongation along the grooves/ridges. Cells are more responsive to groove depth than groove width. Furthermore, cell nuclei also elongate and align on nano-grooves. Besides cellular morphology, cellular functions were also affected by nano-grooved topography. For example, albumin synthesis was enhanced in the hepatocytes that were cultured on the nano-grooved silicon substrates compared to those on the flat surface. We also found that gene transfection via polyethyleneimine carriers was decreased when the cells were cultured on nano-grooved surfaces. Grooved architecture is particularly suitable for investigation of tissues with cellular anisotropic arrangement, such as skeletal muscle, cardiac muscle, tendon, ligament, and nerve. We found that the differentiation of myoblasts into myotubes was enhanced by nano-grooves/ridges. Myogenesis of mesenchymal stem cells was also improved on grooved substrates. Furthermore, by combination of grooved structure with biochemical signals or elastic properties, we could investigate the contribution of different signals on cell behavior. We found that synthesis of type I collagen by anterior cruciate ligament cells was enhanced on the grooved structure that was conjugated with RGD. Similarly, RGD conjugation enhanced the myogenesis of myoblasts on nano-grooved surfaces. By culturing cardiomyocytes on grooved polystyrene or polyurethane, we found that the morphology and orientation of cardiomyocytes were affected mainly by the nanogrooved structures, while the contractile ability of the cells was

Session No.: S21-05

Human Mesenchymal Stem Cell Responses to Hydrostatic Pressure and Shear Stress

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The effects of mechanical stimuli to which cells are exposed in vivo are, at best, incompletely understood; in this respect, gene-level information regarding cell functions which are pertinent to new tissue formation is of special interest and importance in applications such as tissue engineering and tissue regeneration. Motivated by this need, the present study investigated the early responses of human mesenchymal stem cells (hMSCs) to intermittent shear stress (ISS) and to cyclic hydrostatic pressure (CHP) simulating some aspects of the biological milieu in which these cells exist in vivo. Production of nitric oxide (NO) and mRNA expression of several known mechanosensitive genes as well as ERK1/2 activation in the hMSC response to the two mechanical stimuli tested were monitored and compared. NO production depended on the type of the mechanical stimulus to which the hMSCs were exposed and was significantly higher after exposure to ISS than to CHP. At the conditions of NO peak release ((i.e., at 0.7 Pa for ISS and 50,000 Pa for CHP), ISS was more effective than CHP in up-regulating mechanosensitive genes. ERK1/2 was activated by ISS but not by CHP. The present study is the first to report that PGTS2, IER3, EGR1, IGF1, IGFBP1, ITGB1, VEGFA and FGF2 are involved in the response of hMSCs to ISS. These findings establish that, of the two mechanical stimuli tested, ISS is more effective than CHP in triggering expression of genes from hMSCs which are bioactive and pertinent to several cell functions (such as cell differentiation and release of specific growth factors and cytokines) and also to tissue-related processes such as wound healing.

Session No.: S21-06

Regulations of Protein-protein Interaction to Direct Stem Cell Differentiation on Scaffold Surface

Guo-Chung Dong[1], G. Vijaya Narasimha[1], Chi-Han Li[1], Chun-Hao Li[1], Cheng-Hao Wang[1], Tsang-Wen Chang[1], Po-Tsang Huang[1], Ya-Wen Hsiao[1]

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Three elements involved in tissue engineering are cells, scaffolds and signals. Among these, signals and surface characters of scaffolds play important roles in facilitating cell adhesion, migration and differentiation etc. BMP-2 and EGF, bone morphogenetic protein 2 and epidermal growth factor, are two of important signaling proteins. In the beginning of signaling, both of them will bind with their receptor, BMP receptor and EGF receptor, to initiate following signaling transduction. In this study, we try to (1.) prolong BMP and EGF signals through tethering them on surface; (2.) enhance signaling strength through increasing their binding affinity by specific small molecular binders. In the study, BMP-2 and EGF are immobilized respectively on surface with or without TCM synergizing. Molecular binding, signal transduction, cell behaviors and osteo-induction are performed respectively by using SPR, over-expression cell line, bone marrow stromal cells, BMSC and rabbit calvarias defect. In molecular binding, the result showed that in spite of immobilization, BMP-2 and EGF also can maintain the binding activity to BMPR and EGFR. Some of binding enhancer and inhibitor are found from 60 kinds of Traditional Chinese Medicines, TCM. Among 60 of TCMs, Gusuibu and Cortex Moutan Radicis are found to increase the affinity of BMP-2 and EGF binding to receptor, which are through increasing the quantity and association rate, ka, and decrease the dissociation rate, kd, of EGF bind to EGFR. From the aspect of signal transduction, tethered BMP-2 and EGF can stimulate BMPR and EGFR phosphorylation and down-stream signaling in C2C12 and A431 cell line. In the synergizing of TCM, Gusuibu and Cortex Moutan Radicis can enhance phosphorylation in C2C12 and A431. Gusuibu also are found t o increase the proliferation in BMSC and osteoblastic differentiation in C2C12. From the aspect of tissue regeneration, both of tethered BMP-2 and TCM synergizing induce more

Session No.: S21-07

Engineering Three-dimensional Cellular Mechanical Microenvironment with Magnetic Microscale Hydrogels

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Cellular mechanical microenvironment plays a vital role in various tissues including skin, bone, skeleton, and cardiac muscle. Although current approaches for engineering cellular mechanical microenvironment have significantly facilitated the development of tissue engineering, they suffer from several limitations such as limited diffusion ability for large specimen size, low throughput, and limited controllability at biologically relevant microscale. To address these challenges, we present here a method to engineer cellular mechanical microenvironment with magnetic microscale hydrogels (i.e., magnetic microgels). Magnetic hydrogels find widespread applications in biomedical engineering, including tissue engineering, drug delivery/release systems, and soft actuators, due to their improved controllability, actuation and response properties. In this study, magnetic microgels were fabricated using a multi-step lithography method. The influences of UV crosslinking time, magnetic nanoparticle concentration and monomer concentration on microgel fidelity were investigated. Magnetic responsibility of the magnetic microgels and cell encapsulation ability of the fabrication process were both verified, demonstrating the potential of our method for engineering three-dimensional cellular mechanical microenvironment in vitro.

Session No.: S22-01 Keynote Speaker

Bio-Intelectual Biomaterials for Osteoarthritis (OA) Cartilage Repair

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Traumatic rupture and a group of diseases such as osteoarthritis (OA) often result in cartilage degeneration and defects in the knee. The developmental biology of growth plate is of key importance in understanding the molecular pathology of OA, and there is much evidence indicating that signaling pathways modulating endochondral ossification (EO) during growth plate development also affect the pathogenesis of OA. We learned from the developmental biology of growth plate and uncovered the roles of several important signals including Rho GTPases and PTHrP in chondrocytes pathologic changes in vitro as well as in mice OA development. Furthermore, we also integrated biomaterials with bioactive peptide, chemical inhibitors, and metal ion that targeting these signals to facilitate articular cartilage repair and regeneration.1) It was found that the activity of Rac1, one of Rho GTPases was aberrantly elevated in OA chondrocytes. The down-regulation of OCRL1, one of GTPase-activating proteins (GAPs) accounted for Rac1 activation in OA chondrocytes. Moreover, genetic or pharmaceutical modulation of OCRL1-Rac1 axis affected human chondrocytes hypertrophy and calcification in vitro and osteoarthritis development in vivo. 2) Li+ ions enhanced the proliferation and osteogenic differentiation of bone BMSCsthrough activation of the Wnt/β-catenin signalling pathway, besides, Li+ ions protecting chondrocytes and cartilage tissues from the inflammatory OA environment through activation of autophagy.We further incorporated Li+ ions into bioactive MBG scaffolds as a viable strategy for fabricating bi-lineageconducive scaffolds that enhanceregeneration of osteochondral defects. Our studies collectively demonstrate that modulation of important signals in growth plate development in articular cartilage pathologic changes may function as an effective method to develop potential therapeutics for OA and improve cartilage repair efficiency

Session No.: S22-02 Invited Speaker

In Vitro Cartilage Regeneration and Its Clinical Translation

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Cartilage regeneration and its functional reconstruction is always a great challenge in clinical treatment. Tissue engineering provides a new strategy for solving this issue. During the past ten years, our group performed a series of basic research, established a number of critical techniques for in vitro cartilage construction, and finally realized clinical translation of cartilage regeneration technologies. The main advances includes: 1) To establish a novel chondrogenic induction system by mimicking the chondrogenic microenvironment through in vitro co-culture or in vivo co-transplantation of chondrocytes and mesenchymal stem cells, which could efficiently regulate chondrogenesis and cartilage regeneration of stem cells and thus help to solve the problem of seed cell source; 2) To establish a series of technological system of in vitro 3D cartilage regeneration, and to realize accurate shape control of in vitro regenerated cartilage by combining with 3D print techniques; 3）To develop bioreactor to be used specially for cartilage regeneration, which efficiently enhanced the mechanical properties of in vitro regenerated cartilage; 4) To establish and successfully repair various cartilage defect models (such as articular osteochondral defects, tracheal defects, meniscus defects etc.) in large animals; 5) To successfully perform various clinical trials of cartilage regeneration and its functional reconstruction based on in vitro regenerated cartilage, including reconstruction of external ear, repair and reconstruction of nasal cartilage defects, repair of tarsal plate defects, repair of articular cartilage defects as well as repair of articular osteochondral defects.

Session No.: S22-04

Regeneration of Human-ear-shaped Cartilage by Co-culturing Human Microtia Chondrocytes with BMSCs

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Previously we had addressed the issues of shape control / maintenance of in vitro engineered human-ear-shaped cartilage. In the current study, we believed the lack of clinically applicable cell source had become a major concern. Autologous microtia chondrocytes (MCs) and bone marrow stromal cells (BMSCs) were both promising chondrogenic cells that did not involve obvious donor site morbidity. However, limited cell availability of MCs and ectopic ossification of chondrogenically induced BMSCs in the subcutaneous environment greatly restricted their applications in external ear reconstruction. In addressing this issue, the current study demonstrated that MCs possessed strong proliferation ability but accompanied with rapid loss of chondrogenic ability during passage, indicating a poor feasibility to engineer the entire ear using expanded MCs. Fortunately, the co-transplantation results of MCs and BMSCs (25% MCs and 75% BMSCs) demonstrated a strong chondroinductive ability of MCs to promote stable ectopic chondrogenesis of BMSCs in subcutaneous environment. Most importantly, a human ear-shaped cartilaginous tissue with delicate structure and proper elasticity was successfully constructed by seeding the mixed cells (MCs and BMSCs) into the pre-shaped biodegradable ear-scaffold followed by subcutaneous implantation in nude mouse. These results may provide a promising strategy to construct stable ectopic cartilage with stem cells (BMSCs) for autologous external ear reconstruction.

Session No.: S22-05

The Interactions of Tribological Behaviors with Mechanobiological for Cartilage: Set Up an in Vitro Friction Testing of Cartilage

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If a tissue engineered cartilage cannot withstand the external stress, it will change the cell viability and the balance between extracellular matrix synthesis and degradation. This will lead to the degeneration of cartilage tissue. However, it is still unclear that the effects of biomechanical variation caused by tribology on tissue matrix and cell type. This study plans to develop a universal testing platform including macro- and nanotribological characteristics for tissue engineered cartilage. The biomechanical properties of knee articular cartilage obtained from human, porcine and rabbit species will be assessed and verified by the testing platform. In addition, we measure the biomechanical properties of tissue engineered cartilages prepared by various biomaterials including collagen, fibrin glueand HA combined with different culture environments containing 2-D, 3-D and in vivo culture systems. This will provide a useful database for reference and benefit to develop the functional tissue engineered cartilage with excellent mechanical strength. Moreover, articular cartilage tissue is located in an active and tribological joint. One ofthe major functions is to decrease friction and contribute lubrication. As well, the tissue engineered cartilage is purposed to repair the tribological surface of the damaged cartilage. Therefore, we investigate the changes in biomechanical and lubricative behaviors of the tissueunder the stress in a tribological process. The effects of the tribological process on extracellular matrix and tissue performance are also estimated. By the comparative analyses of lubrication and biochemical properties, the mechanobiological interactions and possible mechanisms are also studies. These results could provide a valuable reference for functional tissue engineered cartilage. In this study, we set up an in vitro friction testing of cartilage now, and hope it can be applied in the direction of the above of the use in the future.

Session No.: S23-01 Keynote Speaker

Potential of Cell Sheets for Cartilage Regenerative Therapy

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Introduction: Although regenerative therapies have been used to treat the knee joint for ~20 years, research on osteoarthritis (OA), the main cause of knee problems, is limited and new regenerative therapies are needed Clinical research using autologous cell sheets: Using animal experiments, we investigated the regenerative effects of layered chondrocyte sheets for the two types of cartilage defects found in OA: partial- and full-thickness defects. After completing preclinical studies, we conducted a clinical study of articular cartilage regenerative therapy in which autologous chondrocyte sheets were transplanted into eight patients. All patients have progressed favorably after surgery, and no serious adverse events have been observed. What distinguishes this regenerative therapy from others is that our clinical study was the first in Japan to apply regenerative therapy to patients with OA. We have concluded our clinical study and, as the next step, we are preparing to apply for “Advanced Medical Care” and for pharmaceutical approval of an industry-sponsored clinical trial. Allogeneic chondrocyte sheets: Cartilage is one of the few tissues that does not require immunosuppressants for transplantation. To take advantage of the immunoprivileged nature, we have studied the potential of allogeneic cells for regenerative therapy by exploring cell sources, analyzing their safety and immunogenicity, and developing cryopreservation methods for allogeneic cell sheets. We have begun a clinical study to apply this potentially safe and highly effective regenerative therapy to patients using allogeneic cell sheets. Currently, we are collecting, cryopreserving, and selecting cells suitable for human transplantation.

Session No.: S23-02 Keynote Speaker

Moving Platelet-rich Plasma (PRP) to Bedside and Industry

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With increased utilization of platelet-rich plasma (PRP), it is important for clinicians to understand that blood products such as PRP fall under the prevue of FDA's Center for Biologics Evaluation and Research (CBER). PRP are exempt and therefore do not follow the FDA's traditional regulatory pathway. However, the most concern is PRP preparation systems to the market, in which the devices for PRP preparation should follow Device Class and Regulatory Controls.

We have demonstrated that PRP composed of various growth factors would facilitate the recovery of osteochondrogenic degeneration and defects. PRP has been moved from bench side to bedside in which the phase II clinical trial of PRP for therapy of degenerated human intervertebral disc (IVD) are being currently conducted by our research group in Taipei Medical University Hospital (TMUH) (approved by DOH in Documentation 0980263734). PRP preparation system to the market was also well established. For example, PLTenusTM Platelet Concentrate Separator is designed for the safe and rapid preparation of autologous PRP from blood under the patient’s point of care. Using a blood collection needle, only a small amount of blood (7 ml) was drawn from arm vein into the PLTenusTM tube and gently invert it 3 to 5 times to mix the anticoagulant additive with the patient’s blood. The PLTenusTM were symmetrically inserted into opposite sides of the centrifuge and spun at “Centrifuge mode I” at room temperature for 8 min. After centrifugation, blood components are separated with the pure PRP resting on the separator gel. The supernatant fraction was gently mixed 2 to 3 times and then collected using syringe. High yield and purity of pure PRP, without leukocytes contamination, was ready for clinical treatment.

Session No.: S23-03 Invited Speaker

Autokogous Epithelial Cell Sheet Products for Esophageal Regeneration

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Esophageal stricture remains one of the major problems associated with large endoscopic submucosal dissections (ESD) for superficial esophageal neoplasms. In our previous safety and feasibility study, tissue-engineered epithelial cell sheets, produced by culturing the patients' own oral mucosal epithelial cells on temperature responsive culture dishes, were transplanted onto the ulcer surface following ESD in 10 patients. The result of the study indicate that autologous epithelial cell sheets were reproducibly produced, transplanted safely, and promoted early re-epithelialization. However, the novel approach had several shortcomings. One of the crucial challenges was the cell sheet transplantation technique – to transport and transplant the sheets properly into the esophageal lumen. Encouraged by the clinical results and the challenge regarding delivery, we developed a new endoscopic device using a 3D printer. To prevent losing or damaging the cell sheet during delivery through the oral cavity and pharynx, an applied vacuum can draw the sheet in and protect it by the device's walls. Once at the position of the ulcer surface, pressure increase can expand a membrane with attached cell sheet, thus facilitating rapid, simple, and accurate transplantation. At present, we are planning clinical trials of esophageal regeneration using epithelial cell sheets and dedicated devices as human cell tissue products-based therapy in Japan and Sweden.

Session No.: S23-04 Invited Speaker

Periodontal Regeneration with Autologous Periodontal Ligament-derived Cell Sheets

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Background Periodontitis results in the destruction of tooth-supporting periodontal tissue, and would not heal spontaneously. Various approaches have been introduced to regenerate periodontal tissue, however, the efficacy was limited, especially in severe cases. We studied the transplantation of autologous periodontal ligament-derived (PDL) cell sheets for the regeneration of periodontal tissue in clinical settings. Methods We performed a single-arm and single-institute phase I study to establish the safety and efficacy of PDL cell sheets in patients with periodontitis. Wisdom teeth were extracted, and periodontal tissues were sclaped as cell sources from ten patients with severe, chronic periodontitis, ranged in age from 33 to 63 years (mean [±SD], 46±12). Human cellular products were created in the cell processing center in accordance with GLP and GMP guidelines. Three-layered PDL cell sheets were constructed with temperature-responsive culture dishes and transplanted autologously following standard periodontal surgeries. Bony defects were filled with beta-tricalcium phosphate granules. Cone-beam computed tomography was performed to evaluate the periodontal regeneration at every 6 months. Results All the findings, including reduction of periodontal probing depth, clinical attachment gain, and increase of radiographic bone height, were improved in all patients. These therapeutic effects were sustained during a mean follow-up period of 36 months, and there were no complications. Conclusions The results of this study provide the safety and efficacy of PDL cell sheets. This cytotherapeutic approach might be an alternative strategy to regenerate the periodontium and improve patients' quality of life.

Session No.: S23-05 Invited Speaker

Middle Ear Mucosal Regeneration by Nasal Mucosal Epithelial Cell Sheets Transplantation

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Recurrence of cholesteatoma is mainly caused by poor mucosal regeneration in the middle ear cavity and mastoid cavity, and changes such as granulation tissue formation can occur, which impair gaseous exchange in the middle ear cavity. If middle ear mucosa can be preserved and the rapid postoperative regeneration of mucosa on the exposed bone surface can be achieved after middle ear surgery, surgical treatment for otitis media including cholesteatoma can be potentially improved, and the physiological function of middle ear can be recovered. Conventional canal wall up tympanoplasty often results in a lack of mucosal regeneration in the resected area of the mastoid cavity and changes such as granulation tissue formation occur. In particular, mucosal regeneration in a poorly pneumatized mastoid cavity is extremely difficult. To overcome these limitations, we developed a novel method combining canal wall up tympanoplasty and autologous epithelial cell sheet transplantation for postoperative regeneration of the middle ear mucosa. We obtained the approval of the ethics committee of our institution and the Ministry of Health, Labor, and Welfare. In the clinical research, we endoscopically removed an approximately 10 × 10-mm2 nasal mucosal tissue from her inferior concha. Tissue-engineered autologous nasal mucosal epithelial cell sheets were fabricated by culturing the harvested cells using keratinocyte culture medium (KCM) containing autologous serum for 26 days in an aseptic environment in a good manufacturing practice (GMP)-compliant cell processing center (CPC). The cultivated cell sheets were transplanted, during canal wall up tympanoplasty, onto the exposed bony surface of the attic of the tympanic and mastoid cavities where the mucosa was lost. We have performed this procedure on five patients with middle ear cholesteatoma. All patients showed a favorable postoperative course, with no adverse events or complications.

Session No.: S23-06 Invited Speaker

Present and Future Perspectives on Cell Sheet-based Myocardial Regeneration Therapy

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Heart failure is a life-threatening disorder worldwide and many papers reported about myocardial regeneration through surgical method induced by left ventricular assist device, cellular cardiomyoplasty (cell injection), in situ engineering (scaffold implantation), and left ventricular restrictive devices. Some of these innovated technologies have been introduced to clinical settings. Especially, cell sheet technology has been developed and has already been introduced to clinical situation. The myoblast sheets have been established and these sheets have proved to secrete multiple cytokines such as HGF or VEGF in vitro study. Moreover, in vivo studies using large and small animal heart failure model have been done and myoblast sheets could improve diastolic and systolic performance by cytokine paracrine effect such as angiogenesis, antifibrosis, and stem cell migration. Recently evidenced by these preclinical results, clinical trial using autologous myoblast sheets has been completed in ischemic cardiomyopathy patients and some patients showed left ventricular reverse remodelling, improved symptoms, and exercise tolerance. Autologous skeletal myoblast sheets (HeartSheet®) are the first cellular or tissue-based product for treating severe heart failure to be approved for manufacture and sale in Japan. Recent works demonstrated that iPS cell-derived cardiomyocyte sheet were developed and showed electrical and microstructural homogeneity of heart tissue in vitro, leading to the establishment of proof of concept in small and large animal heart failure model.

Session No.: S24-02 Keynote Speaker

Crosstalk Between Integrin-Linked Kinase (ILK) and Wnt Signaling Pathway Conveys Surviving Signals from Epithelial Basement Membrane to Keratinocyte Stem Cells

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It has been well known that stem cell differentiation is heavily influenced by the microenvironment, so called the stem cell niche. For epidermal keratinocytes, epithelial basement membrane (EBM) is one of the major components of the niche. In the eye, the niche for the corneal epithelial stem cells is located at the EBM zone of the limbus. Although the ultrastructures and matrix components of the limbal EBM zone have been extensively studied, to date little is known about how the surviving signal is transmitted from the limbal extracellular matrix (ECM) to the epithelial stem cells. Previously, we reported using EDC/NHS to cross-link de-epithelialized amniotic membrane (CLDAM) to fabricate a novel scaffold for cultivation and transplantation of human limbo-corneal epithelial (HLE) cells (Biomaterials, 2010). Later, using CLDAM as a surrogate EBM, we were able to demonstrate that HLE progenitor cells were better preserved on CLDAM than on natural DAM (J. Biomed. Nanotechnol., 2013). Recently, we proposed that integrin-linked kinase (ILK) may play a key role in conveying surviving signals from ECM and cross-talking with the Wnt pathway so as to promote the proliferation and inhibit differentiation of HLE cells. We showed that rougher surface of CLDAM better activated ILK, and by inhibiting GSK3β, activated the Wnt pathway. Activation of the Wnt in turn up-regulated the expression of p63, the master regulator for corneal epithelial stem cells (Acta Biomaterialia, 2016). Because non-organic material with adequate surface complexity can relay survival or differentiation signals to cells, the three-dimensional configuration of the stem cell niche might be more important than previously recognized. Understanding the topographic features of individual SC niches may enable the fabrication of artificial SC niches using synthetic biomaterials to replace more expensive natural basement membrane proteins.

Session No.: S24-06 Keynote Speaker

Reviews for Clinical Trials of Human Pluripotent Stem Cells for Ocular Diseases and Human Pluripotent Stem Cell Culture on Xeno-free Conditions

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Human pluripotent stem cells (hPSCs) hold excellent promise for regenerative medicine and drug discovery from their high differentiation ability into any kind of cell types in human tissues. However, clinical application of hPSCs is currently extremely limited compared to the clinical application of adult stem cells. Ocular diseases are the first human trials of hPSCs and Phase I/II trials showed promising safety results as well as some possible efficacy. The current clinical trials evaluating hPSC-based therapies predominantly targeting on treatment of retinal degeneration in the eye. This is because eye tissue has an immunoprivileged nature (tolerating characteristics of foreign antigens and non-histocompatible cells; almost no immune response to foreign materials). It can be possible to visualize the internal tissue through lens after transplantation of the cells. Age-related macular degeneration (AMD) and Stargard macular dystrophy are the progressive degradation of light-sensing photoreceptor cells and their supportive retinal pigment epithelium (RPE). Preclinical investigation showed the safety and efficacy of hPSC-derived RPE in animal models. Several clinical trials are now ongoing for hPSC-derived RPEs for AMD and Stargard macular dystrophy. The current status of clinical trials of hPSC-derived RPE for ocular diseases is reviewed in this study. Furthermore, hPSC culture on biomaterials grafted with nanosegment are discussed for future clinical usage of hPSCs for treatment of ocular and other disease.

Session No.: S24-01 Keynote Speaker

Biomaterials Induced Corneal Regeneration: Bench to Bedside and Back

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Biomaterials based on collagen, the major extracellular matrix (ECM) component of the human body have been successfully stimulating tissue regeneration in clinical trials. Collagen derived from animal or human cadaveric sources, however, suffer from batch to batch heterogeneity and carry a risk of pathogen transmission. These problems are circumvented by using recombinant human collagen (RHC). We have shown that cell-free cornea implants made from RHC can stimulate the regeneration of corneal epithelium, stroma and nerves in 10 patients in a clinical trial without the need for immune suppression beyond a short course of prophyllactic eye drops. In patients with severe pathologies such as chemical burns or previous graft rejections, who are at a high risk for rejection of conventional human donor corneas, however, we developed interpenetrating networks of RHC and a synthetic phosphorylcholine. Addition of the second network to the RHC allowed for implants to achieve stable restoration of corneal integrity in all patients with corneal ulcers and surface erosions tested. In a proportion of patients who lacked stem cells and had conjunctival invasion, however, these individuals will require a follow-up corneal limbal stem cell transplantation. Nevertheless, the implants have sufficiently restored the underlying stroma to support a stem cell graft. Like native collagen, however, RHC is a large and relatively chemically biopolymers, leading to the development of a range of collagen-like peptides (CLPs) or collagen mimetic peptides (CMPs) as collagen analogs. We compared the versatility and functionality of one such CLP to that of full-length collagen as scaffolds for promoting regeneration in vivo in mini-pig and rabbit models. We also showed that CLP-based hydrogels affected cell differentiation and regeneration by stimulating host cells to effect the regeneration by recreating the ECM complement of the normal cornea.

Session No.: S24-03 Invited Speaker

Suppression of Hippo Pathway by Lysophosphatidic Acid in a Novel Proliferative Corneal Organ Culture System

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Previously, we have shown that addition of lysophosphatidic acid (LPA) results in expression of YAP, a core protein in the Hippo pathway, and then proliferation in contact-inhibited corneal endothelial cell (CEC) monolayers without change in normal phenotypes. Here, we aim to investigate the effect of LPA on CEC proliferation or density in a proliferative corneal organ culture system. Briefly, rabbit corneas were excised and cultured for 7 days in a modified corneal organ culture medium (MEM+10% FBS). Corneas were treated by PBS, Y27632 (a ROCK inhibitor), siRNA to p120-catenin, or LPA from Day 2 on. Cell density and central corneal thickness were measured. Samples were then fixed, paraffin-embedded and examined morphologically by HE-staining and by imunofluorescent staining of ZO-1, p120, YAP, SMA and BrdU labeling. MTT assay was performed to detect signs of cytotoxicity. Consequently, phase-contrast microcopy revealed endothelial loss and signs of apoptosis after 72h of organ culture, but no CEC alterations in cultures added with LPA. From Day 3 to Day 7 the cell density was significantly more while the central corneal thickness was less in cultures added with LPA. HE-staining failed to demonstrate significant differences between the four groups. Imunofluorescent staining displayed similar patterns of ZO-1 and SMA, but enhanced nuclear staining of YAP and BrdU labeling in the LPA gruop. MTT assay detected signs of cytotoxicity in the control and Y27632 but not the YAP group. In summary, a stable organ culture system for proliferation can be established, in which suppression of Hippo pathway by adding LPA results in enhanced CEC proliferation and increased cell density. This novel organ culture protocol may be applied to eye banking, to optimize corneal grafting and to contribute to regenerative medicine.

Session No.: S24-04 Invited Speaker

Development of Functional Human Oral Mucosal Epithelial Stem Cell Sheets Using a Defined, Feeder-free and Serum-free Culture System for Ocular Surface Reconstruction

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Ocular surface reconstruction (OSR) using tissue-engineered cultivated oral mucosal epithelial cell sheets (COMECS) is a promising newly developed treatment for patients with severe ocular surface disease. Until now, this technique has used exogenic and undefined components such as mouse-derived 3T3 feeder cells and fetal bovine serum. To minimize associated risks of zoonotic infection or transmission of unknown pathogens and so establish a safe and effective protocol for the next generation of treatment modality, we developed a novel technique for the COMECS protocol, using a feeder-free and serum-free (FFSF) culture system. Following this new protocol, COMECS exhibited 4–5 layers of stratified, well-differentiated cells, and we successfully generated functional COMECS that included holoclone-type stem cells. Immunohistochemistry confirmed the presence of markers for cell junction (ZO1, Desmoplakin), basement membrane assembly (Collagen 7, Laminin 5), differentiation (K13, K3), proliferation (Ki67) and stem/progenitor cells (p75) in the FFSF COMECS. When transplanted to the ocular surfaces of rabbits, the tissue survived for up to 2 weeks. This study represents a first step toward assessing the development of functional FFSF COMECS for safe and ideal OSR.

Session No.: S24-05 Invited Speaker

Effect of Corneal Guttata on Tissue Engineered Endothelial Cell Therapy

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The most common indication for corneal transplantation is a dysfunctional corneal endothelium due to Fuchs' endothelial dystrophy (FED). A diagnostic clinical sign of FED is the presence of excrescences on Descemet membrane (DM), called corneal guttata. Minimally invasive corneal endothelial cell regenerative procedures such as endothelial cell injection therapy and Rho-kinase inhibitor pharmacotherapy have been proposed as alternatives to conventional corneal transplantation for FED patients. However, the effect of guttata on cell migration and monolayer reformation is unknown. Investigations of endothelial monolayer formation on guttata are not possible in vivo, due to lack of suitable animal model. Hence in order to understand the efficacy of these treatments, ex-vivo models are the only option. Based on the clinical observation of guttata structures, we fabricated an in vitro synthetic-guttata model to mimic the topographical micro-environment of FED patients and systematically investigated the migration of primary human corneal endothelial cells and monolayer regeneration on synthetic-guttata. The monolayer formation, which would correlate to the recovery of the corneal endothelial, was found to be significant affected by the density, height and curvature of the guttata-like micro-pillar array. The human corneal endothelial cells were unable to form a monolayer either following the cell injection model or the pharmacotherapy model, on densely-packed synthetic-guttata mimicking late stage FED. The Euclidean distance and directness of cell migration were also significantly lower on densely-packed synthetic-guttata as compared to guttata-free surface. However, the cells could form monolayer and express tight-junctional protein zona occuludin-1 on sparsely-spaced synthetic-guttata of lower height and curved sidewalls, which mimicked the early stage FED. These results suggest that advanced stage FED guttata could hinder corneal endothelial monolayer formation. Hence surgical removal of the most densely-formed corneal guttata combined with the cell regenerative therapy would improve the efficacy of this treatment.

Session No.: S25-01 Keynote Speaker

An Injectable Conductive Polymer Hydrogel Improves Electrical Conduction Velocity in the Injured Heart

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Coronary heart disease is the leading cause of death in the world and new treatments are required to prevent progressive heart failure after a myocardial infarction (MI). Scar formation after an MI delays electrical impulse propagation, inducing dysynchronous cardiac activation and uncoordinated contraction. We generated an injectable biocompatible conductive polymer that not only stabilized the infarct region, but also facilitated electrical signal propagation across the scar tissue to enhance synchronous ventricular contraction. Using oxidative polymerization technique we conjugated polypyrrole (PPY) to chitosan (CHI) and hydrogels were created by glutaraldehyde crosslinking. Electrical conductivity was measured by two-probe cyclic voltammetry. The conductive biomaterial (CHI-PPY) had significantly greater conductivity. Improved electrical propagation was demonstrated when neonatal rat cardiomyocytes cultured on the CHI-PPY and the biomaterial had significantly higher Ca2+ transient propagation velocity (by optical mapping with a fluo-4/AM indicator). When the CHI-PPY was employed for in vivo studies employing MI model, saline, CHI, and CHI-PPY were injected into adult rat hearts 1 week after injury. The sequence of cardiac electrical activation was visualized using an optical mapping system 1 month post-injection. Saline- and CHI-injected hearts showed disrupted propagation patterns and significantly reduced conduction velocity, while CHI-PPY-treated hearts had higher conduction velocities that were similar to healthy controls. In conclusion: A polypyrrole-conjugated chitosan hydrogel supported cell attachment and improved electrical conductivity in vitro. The biomaterial enhanced Ca2+ transient propagation in cultured cardiomyocytes and intra-myocardial injection of the material improved longitudinal electrical impulse propagation across the scar. This new biomaterial may be a new potential therapy to synchronize cardiac contraction.

Session No.: S25-02 Keynote Speaker

Advances in Constructing Human Cardiac Tissue from Stem Cells Including Electrical Stimulation

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Cardiac tissue engineering, particularly that utilising autologous human stem cells, has the potential to produce constructs of cardiac tissue for surgical replacement of inefficient or damaged cardiac muscle or pacemaker tissue: both pediatric and adult applications may be possible. Such constructs could also be useful for drug and pharmacological safety testing platforms. We have established platforms to grow robust cardiac constructs with integrated vascularisation that grow and survive transplantation. Fully vascularised, robust beating cardiac tissue has been grown in pedicles constructed in polyacrylate chambers, implanted in vivo in rats. Both mesenchymal stem cells (MSC) and induced pluripotent stem (iPS) cells can serve as sources of human cardiomyocytes to grow cardiac constructs in vivo. Using the traditional embryoid body (EB) approach from iPS cells, we enhanced the efficiency of cardiac differentiation by the judicious use of the HDAC inhibitor trichostatin-A, along with activin A and BMP4. We are now stimulating differentiating cells electrically to improve differentiation and maturation pathways. These cells in fibrin matrix have been grown into cardiac constructs: iPS cell-derived cell clusters develop contracting constructs in the chamber, incorporating human cardiac cells vascularised by host vessels. Importantly, these human-derived cardiomyocytes survived 4 weeks after implantation showing strong contractile activity and troponin-T striations in the constructs. We have also preconditioned constructs and cells with brief periods of ischaemia before implantation to improve survival. Finally, stimulating EBs electrically for prolonged periods (at 1Hz up to 7 days) in vitro increases the maturation of cardiomyocytes towards an adult ventricular phenotype. Modified bionic chambers have been developed to electrically stimulate developing constructs in vivo for up to 4 weeks, and the outcome of these studies will be discussed. These tissue engineering approaches incorporating endogenous vascularisation provide proof-of-principle for generation of substantial cardiac tissue from human iPS cells.

Session No.: S25-03 Keynote Speaker

Pulsatile Myocardial Tube Fabrication Using Cell Sheet Technology

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We have developed cell sheet-based tissue engineering and realized three-dimensional (3-D) pulsatile cardiac tissues by stacking cell sheets. Layered cardiac cell sheets synchronize electrically and beat macroscopically both in vitro and in vivo. However, critical issue for fabricating thick cell-dense tissues is insufficient supply of oxygen and nutirition, and waste accumulation due to the lack of vascular structure. To clear this problem, multi-step transplantation of triple-layer cardiac cell sheets co-cultured with endothelial cells has been performed. Co-cultured endothelial cells form perfusable blood vessels connecting to host blood vessels in the triple-layer constructs and repeating procedures realized gradual vascular structure fabrication and thick vascularized cardiac tissues in vivo. Furthermore, for in vitro perfusable blood vessel formation within 3D tissues, native tissues with a connectable artery and vein network or artificial collagen gel including perfusable micro channels were prepared as vascular beds and the beds were perfused by using bioreactor systems. Then triple-layer co-cultured cell sheets were transplant on the vascular beds repeatedly. Functional blood vessels connected between the beds and cell sheets and media reached cell sheets. Moreover, repeating procedure realized in vitro vascularized 3-D tissues. As a next challenge, we have tried to fabricate human functional myocardial tubes by wrapping human iPS cell-derived cardiac cell sheets. Myocardial tubes evoke significant inner pressure both in vitro and in vivo. Now the scaling-up of pulsatile myocardial tube are on going by combining with vascularization technologies. Cell sheet-based technology has revealed great potential to fabricate 3-D tissues and organs, and should contribute to future treatments of severe diseases and human tissue model production.

Session No.: S25-04

Microfabrication of Cardiac Fibrosis Model and the Anti-fibrotic Effect of Human Adipose-derived Stem Cells

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Human mesenchymal stem cells (hMSCs) hold great promise in cardiac fibrosis therapy, due to their potential ability of inhibiting cardiac myofibroblast differentiation (a hallmark of cardiac fibrosis). However, the mechanism involved in their effects remains elusive. To explore this, it is necessary to develop an in vitro cardiac fibrosis model that incorporates pore size and native tissue-mimicking matrix stiffness, which may regulate cardiac myofibroblast differentiation. In the present study, collagen coated polyacrylamide hydrogel substrates were fabricated, in which the pore size was adjusted without altering the matrix stiffness. Stiffness is shown to regulate cardiac myofibroblast differentiation independently of pore size. Substrate at a stiffness of 30 kPa, which mimics the stiffness of native fibrotic cardiac tissue, was found to induce cardiac myofibroblast differentiation to create in vitro cardiac fibrosis model. Conditioned medium of hMSCs was applied to the model to determine its role and inhibitory mechanism on cardiac myofibroblast differentiation. It was found that hMSCs secrete hepatocyte growth factor (HGF) to inhibit cardiac myofibroblast differentiation via downregulation of angiotensin II type 1 receptor (AT1R) and upregulation of Smad7. These findings would aid in establishment of the therapeutic use of hMSCs in cardiac fibrosis therapy in future.

Session No.: S25-05

Contractile Force Measurement of Human iPS Cell-derived Myocardial Sheets for in Vitro Drug Testing

Daisuke Sasaki[1], Katsuhisa Matsuura[1], Hiroyoshi Seta[1], Yuji Haraguchi[1], Teruo Okano[1], Tatsuya Shimizu[1]

[1]Tokyo Women's Medical University

We have developed temperature-responsive cell culture surfaces on which cells can adhere at 37 ℃ but cannot adhere below 32 ℃. When cells are cultured and reach confluence on this surface, we can harvest a cell sheet from the surface without any cell damage only by decreasing temperature. This cell sheet technology is highly valuable in creating cell-dense tissues similar to living tissues. Utilizing this technology, in the present study, we created human iPS cell-derived myocardial sheets and investigated their contractile properties. We used a human iPS cell line in which a cardiac α-myosin heavy chain promoter-driven puromycin resistance gene was introduced. Cardiac differentiation of human iPS cells was induced by a bioreactor-based method developed previously [1]. The differentiated cardiomyocytes were enriched by puromycin selection and plated onto temperature-responsive culture dishes. When the cells reached confluence, a fibrin gel sheet was put on the cells and temperature was decreased to 20 ℃, which allowed the myocardial sheet to transfer from the temperature-responsive surface to the surface of fibrin gel sheet. The myocardial sheet with fibrin gel sheet was mounted to a force-measuring device equipped with a force transducer, and the contractile force due to the beating was measured successfully. We confirmed that the contractile force significantly increased due to adrenergic stimulation, and weakened due to the addition of cardiotoxic drugs such as doxorubicin. In conclusion, the present method is useful for the evaluation of myocardial sheet contractility and for in vitro drug testing. References: 1. Matsuura K et al. Biochem Biophys Res Commun 425, 321, 2012.

Session No.: S25-06

Evaluation of Synthetic Vascular Grafts in a Mouse Carotid Grafting Model

Alex Chan[1], Richard P. Tan[1], Praveesuda L. Michael[1], Juichien Hung[1], Laura Z. Vanags[1], Christina A. Bursill[1], Steven G. Wise[1]

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Rationale: There are currently no effective synthetic conduits for small diameter vascular grafting. Current pre-clinical models of graft performance are limited by the necessity for long time points (rat) and prohibitive cost (sheep). A new low cost, high-throughput model of vascular grafting that enabled assessment of novel conduits, including in the context of altered genetic backgrounds would add significant value. Objective: We sought to develop a mouse carotid grafting model, establishing appropriate time points and exemplifying the utility of transgenic strains, to assess the performance of synthetic vascular grafts. Methods & Results: Polycaprolactone (PCL) small diameter (0.5mm×6mm) conduits were produced by electrospinning. PCL grafts were implanted into the carotid artery of C57BL/6 mice (~24g) using an established cuff technique. Grafts were explanted at 7, 14 and 28 days for histopathological analysis. Haematoxylin and eosin staining showed that neointimal hyperplasia increased over time, as a percentage of total lumen area from 26.9±4.5% at day 7, to 44.4±4.7% at day 14 and 60.3±5.1 % at day 28. SMCα-actin, a marker of smooth muscle cell maturity, also increased with time up to 19.3±3.5% of neointimal area at day 28, indicating increasing hyperplasia stability. CD31 immunohistochemistry showed that endothelial cells were not detected in the mid-graft at day 7. By day 14, migration along the graft had increased (40.5±4.9%) and re-endothelialisation was near complete by 28 days (91.3±3.4%). GFP-labelled bone marrow mononuclear cells from FVB-L2G mice were injected intravenously into FBV mice and were identified around the graft at each time point. Conclusions: We have developed a mouse carotid grafting model which develops significant neointimal hyperplasia and re-endothelialises to near completion by 28 days. GFP-labelled bone marrow mononuclear cells were tracked to the graft site following IV injection. Together, our model provides a powerful new tool for high-throughput investigation of new vascular conduits.

Session No.: S26-01 Keynote Speaker

Bioinspired Multifunctional Supramolecular Dendritic Systems as Efficient Theranostic Nanoplatforms for Tumor Treatment

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Bioinspired Multifunctional Supramolecular Dendritic Systems as Efficient Theranostic Nanoplatforms for Tumor Treatment Zhongwei Gu National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China zwgu@scu.edu.cn In recent years, supramolecular self-assembly of dendrimers emerges as a promising strategy for developing a new generation of nanomaterials for theranostic delivery. With the rational molecular and supramolecular engineering, we fabricated dendritic peptide-based mimics of viral architectures and infections for overcoming key biological barriers and navigating systemic delivery, which are able to resist protein adsorption, prolong blood circulation time, as well as provide site-specific drug delivery by passive targeting, extracellular targeting, receptor-mediated active targeting and subcellular targeting. More importantly, capsid-like supramolecular dendritic systems could realize deep tumor drug penetration. In vitro and in vivo results confirmed the multifunctional viral mimics provided much better tumor treatment effects as compared with positive control group, including the aspects of tumor growth inhibition, side effects, and tumor metastasis. On the other hand, molecular probe could be readily incorporated into supramolecular dendritic systems for tumor diagnosis. We built a dual-responsive supramolecular PEGylated dendritic system which was integrated fluorescent probe after disulfide polymerization for efficient platinum-based drug delivery and near-infrared (NIR) tracking. We also set up a supramolecular hybrid strategy to generate supramolecular hybrid dendritic systems with highly efficient gene delivery efficiency in vitro and in vivo owing to the arginine-rich surface of the dendritic-peptide coronas mimicking viral capsids. This hybrid strategy gives some unique features based on inorganic core of quantum dot for intracellular tracking, protein expression monitoring and in vivo bio-imaging, which contribute to the comprehensive understanding of gene delivery pathways and the improvement of delivery systems. We foresee that these works will open a new avenue for the design of more multifunctional supramolecular hybrid dendrimers and additional biomedical applications.

Session No.: S26-02 Keynote Speaker

Gene Transfer Strategies to Promote Chondrogenesis and Cartilage

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Various transgene and gene transfer methods have been used to enhance chondrogenic differentiation and hypertrophy in adult stem cells and to reduce dedifferentiation in culture expanded articular chondrocytes. The current challenge for the clinical applications of gene modified cells is ensuring the safety of gene therapy while guaranteeing the effectiveness, namely, therapeutic levels of translated factors. Viral gene delivery methods have been mainstays in reported literatures. Enhanced safety features recently have shown great potential for clinical application of such technologies. On the other hand, efficiency has been greatly improved in nonviral delivery, which also includes the use of various scaffold materials. Considering that cartilage defect is nonlethal disease unlike genetic disease such as enzyme deficiency, the safety issue take precedence over efficiency issue. In this regard, nonviral delivery method is likely to be prevailing mode of clinical application for enhancing chondrogenesis, provided that the efficiency of nonviral gene transfer closely catch up with those of viral gene transfer. It is also not determined how long the transgene should be expressed to prevent unnecessary events. Future investigations are warranted to provide answers to these questions and finely tune the transfer technologies. ACKNOWLEDGEMENTS:This work was supported by a grant from the National Research Foundation (NRF) funded by the Korean government (2015R1A2A1A09002793)

Session No.: S26-03 Invited Speaker

Polysaccharide-based Nanomedicine for Stimuli-triggered Drug Delivery and Biomedical Imaging

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Polysaccharides, ubiquitous biopolymers made up of repeated mono- or di-saccharides linked via glycosidic bonds, hold great promise as drug carriers because of their unique physicochemical and biological properties, including biocompatibility, biodegradability, and low immunogenicity. In particular, most polysaccharides possess diverse reactive functional groups such as hydroxyls, amines, and carboxyls on their backbones. These groups can be chemically modified to obtain polysaccharide derivatives with unique properties for specific applications. In our group, various nano-conjugates and nanoparticles, responsive to cancer-specific stimuli, have been investigated as the carrier of drug or imaing agent for cancer therapy. As an example, hyaluronica acid (HA)-based nanoparticles were preapred by using an amphiphilic HA derivative as the drug carrier. When such nanoparticles are administered into tumor-bearing mice, they selectively accumulated into the tumor site. Their in vivo tumor targetability were achieved via passive or active targeting mechanism. Once they reach the tumor site, the drug was rapidly released, primarily owing to the characteristic stimuli of tumor such as the low pH, the reductive envionmnent, and the hypoxic condition. Overall, the stimuli-sensitive polysaccharide nanoparticles might have promising potential for cancer theranostics.

Session No.: S26-04 Invited Speaker

Polyplex Namomicelles Assembled with Neprilysin Mrna Augmented Clearance of Beta-Amyloid Peptide from Intracerebroventricular Infusion

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Gene therapy using mRNA is a promising alternative with several advantages over that of plasmid DNA (pDNA); mRNA would be simply translating proteins in cytoplasm without any risk of genome integration. In this study, we administered mRNA encoded for neprilysin (NEP) using polyplex nanomicelles in the A intracerebroventricular (i.c.v.) pre-supplemented model. Since A is the major component of neuritic plaques that often accumulate in the brains of Alzheimer's Disease (AD) patients and NEP play the major role for the clearance of A in brain. As a proof-of-concept study to prove the feasibility of mRNA for treating AD, we constructed a mouse NEP-expressing mRNA and evaluated the capacity for degrading A . The NEP mRNA exhibited superior NEP activity attributed to an apparent capability on the A and human amyloid precursor protein (hAPP) degradation in primary neurons and hAPP overexpressed cells. Furthermore, we evaluated in vivo activity of NEP-expressing mRNA by i.c.v. infusion of the mRNA using polyplex nanomicelles, which allows efficient mRNA introduction in vivo into neural tissues and other various organs. ELISA evaluation revealed that the mRNA injection significantly augmented the NEP level in the mouse brain and effectively reduced the pre-supplemented A in the brain. Meanwhile, a chimeric NEP mRNA engineered to express reporter green fluorescent protein (GFP) simultaneously, showed polyplex nanomicelles successfully delivered NEP mRNA into neurons in vivo. Collectively, mRNA administration is promising to be a new therapeutic approach for neurological diseases such as AD.

Session No.: S26-05 Invited Speaker

Photodynamic Activation of Tumor-targeted Ros-degradable Polymeric Micelles

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In vivo tumor-targeted delivery of nanoparticles encapsulating anti-cancer therapeutics has been pursued for decades to minimize the side effects of cancer chemotherapy. Despite their impressive effects in vitro, limited in vivo benefits have hindered their clinical applications. Well-designed targeting and controlled release capabilities of nanoparticles are often compromised because of complex in vivo situations. Here, we propose a new concept of remote activation of cancer-targeted nanoparticles to increase anti-cancer therapeutics in vivo by implementing photodynamic effects within reactive oxygen species (ROS)-degradable polythioketal nanoparticles incorporating photosensitizers. The polythioketal-based polymer micelles were fabricated from the self-assembly of the amphiphilic block copolymer of poly(1,4-phenyleneacetone dimethylene thioketal) (PPADT) and polyethylene glycol (PEG). TPP and paclitaxel were encapsulated within PEG-b-PPADT micelles, whereby folic acid was decorated on the surface of the micelles by incorporating folic acid-PEG-b-PPADT. The light-induced degradation of the micelles was confirmed using NMR and GPC. HeLa cells were treated with the micelles at various concentrations of paclitaxel and exposed to visible light illumination (650 nm, 70 mW cm-2) for 20 min. The intravenous injections of the micelles with a low paclitaxel dosage (1 mg kg-1), followed by visible light illumination on tumor sites were performed. Localized irradiation of visible light to the nanoparticles targeted to tumor sites selectively activates boosted release of anti-cancer drugs. Studies using a xenograft tumor mouse model demonstrated that the in vivo therapeutic effects are very efficiently controlled by light illumination. Our study suggests that the ROS-sensitive degradable polymeric nanoparticles can be used as a new promising platform for light-controlled delivery of anti-cancer therapeutics. Acknowledgements: This study was supported by the Korean Health 21 R&D Project of the Ministry of Health & Welfare, Republic of Korea (A111552).

Session No.: S26-06

Synthesis of Magnesium Silicate Hollow Nanospheres as High-performance Drug Carriers

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Among the magnesium-containing materials, magnesium silicate nanostructured materials are expected to be excellent biomaterials for applications in various biomedical fields owing to their good biocompatibility, high affinity with guest molecules, and high specific surface area. Magnesium silicate materials were investigated for the adsorption of heavy metal ions and organic pollutants. However, the applications of magnesium silicate nanostructured materials in drug delivery have been less reported. Moreover, the nanostructured materials with a hollow structure have drawn intense interest over the past decades owing to their unique hollow structure, high specific surface areas, and low density. Hence, magnesium silicate hollow nanospheres (MSHNSs) with good biocompatibility, high specific surface area and high loading capacity for drugs offer promising prospects for their applications in drug delivery. Herein, we have successfully prepared magnesium silicate hollow nanospheres using a classical Stöber method in combination of a template based solvothermal process. The as-prepared MSHNSs with sizes of ~110 nm have an ultrahigh specific surface area of 585.6 m2 g−1, as well as high doxorubicin (DOX) drug loading capacity (559 mg g−1). Moreover, the DOX loaded MSHNSs (MSHNS/DOX) exhibits a sustained and pH-responsive drug release performance, and shows a higher anticancer activity than free DOX in vitro. The as-prepared MSHNS/DOX drug delivery system can enter the cytoplasm of human gastric carcinoma (MGC-803) cells and release DOX into the cell nuclei to kill the cancer cells. Considering the advantages such as ultrahigh specific surface area, high biocompatibility, good degradability and high anticancer drug loading capacity, the MSHNSs are promising for the applications in various biomedical fields such as anticancer treatment.

Session No.: S26-07

An Integrated Double-filtration Device for Detection of Exosomes from Urine for Bladder Cancer Diagnosis

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Exosomes are present in a variety of bodily fluids and they play an important role in cellular communication. Studies have shown that the number of exosomes and exosome-associated biomarkers (proteins, nucleic acids, and lipids) can be used to aid clinical diagnosis. Although ultracentrifugation is commonly used for exosome isolation, it is not practical for clinical settings. Here, we developed an integrated double-filtration device that isolated and enriched exosomes from urine, and subsequently detected/quantified exosomes from urine via microchip ELISA. Results showed that that the concentration of exosomes was significantly elevated compared to healthy controls. Receiver operating characteristic analysis demonstrated that this integrated exosome quantification device had a sensitivity of 81.3% at a specificity of 90% (16 bladder cancer patients and 8 healthy controls). Thus, this integrated device shows great potential to supplement urine cytology for diagnosis of bladder cancer in point-of-care (POC) settings.

Session No.: S27-01 Keynote Speaker

Continuous Harvest and Expansion of Human Stem Cells on Thermoresponsive Nanobrush Surface

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Stem cells, such as human adult stem cells (hASCs), human embryonic stem cells (hESCs), and induced pluripotent stem cells (hiPSCs), are attractive prospects for regenerative medicine, translational medicine, and drug discovery. However, hESCs and hiPSCs should be cultured in specific environmental conditions, such as on mouse embryonic fibroblasts, Matrigel, or specific biomaterials immobilized by extracellular matrix (ECM) and ECM-derived oligopeptides, to keep their pluripotency because hESCs and hiPSCs are easy to differentiate and cannot be cultured on conventional culture dishes and microcarriers. Furthermore, common stem cell culture is based on batch-type culture, which is laborious and expensive. Here, we propose a continuous harvest method of stem cells cultured on thermoresponsive nanobrush surfaces where stem cells are partially detached by decreasing the temperature of the culture medium to be lower than the critical solution temperature on the thermoresponsive surface and continuously culturing the remaining cells for their expansion in a fresh culture medium at 37 ˚C. The detached stem cells can be harvested in an exchanged culture medium after their detachment from the thermoresponsive nanobrush surface. The thermoresponsive nanobrush surface was prepared by coating block copolymers containing polystyrene (hydrophobic anchoring domain on dishes) and three types of polymers: (a) polyacrylic acid for cell-binding oligopeptides, (b) thermoresponsive poly-N-isopropylacrylamide, and (c) hydrophilic poly(ethleneglycol)methacrylate. The optimal length and coating composition of these copolymers for adequate attachment and detachment of human adipose-derived stem cells (hADSCs) and embryonic stem cells (hESCs) were determined. A total of 5 cycles and 3 cycles of continuous harvest of hADSCs and hESCs, respectively, on the thermoresponsive nanobrush surface by partial detachment of the stem cells from the surface were demonstrated. Such continuous harvest of stem cells should downsize the equipment requirements for stem cell culture and simplify the culture process.

Session No.: S27-02 Keynote Speaker

Enhanced Regenerative Capabilities of Adipose-derived Stem Cells in Scaffold-free 3D Culture Systems

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Adipose-derived stem cell (ASC) is a valuable source of cell therapy, and we aimed to explore the regenerative capabilities of ASC in scaffold-free three-dimensional (3D) culture conditions. We manipulated spheroid formation of human ASCs by culturing them on chitosan films, and we also stimulated extracellular matrix (ECM) secretion of ASCs and fabricated cell sheets by treatment with ascorbate 2-phosphate (A2-P). Enhanced expression of stemness markers Sox-2, Oct-4 and Nanog was noted in ASCs within cell spheroids and sheets, with significantly enhanced neurogenic and hepatogenic transdifferentiation capabilities relative to monolayer ASCs. Meanwhile, adipogenic and osteogenic differentiation capacities of ASCs were still maintained. The spheroid-derived ASCs expressed significantly more angiogenic growth factors comparing to monolayer ASCs. The interaction between stromal-derived factor-1 and CXCR4 plays an important role in the homing of ASCs to the site of injury, and we found upregulation of CXCR4 in the spheroid-derived ASCs. The enhanced expression of CXCR4 was associated with enhanced proliferation, reduced apoptosis and increased expression of matrix metalloproteinases. Moreover, ASC treatment with A2-P and collagen synthesis inhibitors significantly inhibited the A2-P-enhanced expression of stemness markers. These findings demonstrated that A2-P enhances stemness of ASCs through ECM synthesis in the 3D culture systems. We also found that A2-P-stimulated ECM synthesis in ASCs may be mediated through ERK1/2 pathway. Using a murine model of healing-impaired cutaneous wound, faster wound healing was noted in the group that received ASC spheroid or sheet treatment, and we observed significantly more engrafted ASCs with evidence of differentiation toward endothelial and epidermal lineages in the wound tissue. In summary, biomaterial modulation of ASCs for spheroid formation and A2-P-induced ASC sheet fabrication represent promising approaches of enhancing their regenerative capabilities. The mechanism could be attributed to the recreation of a stem cell niche in vitro.

Session No.: S27-06 Keynote Speaker

Role of Mechanics in Mesenchymal Stem Cell Chondrogenesis

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The unique properties of mesenchymal stem cells (MSCs) and their natural presence within the bone marrow make them an attractive source of cells for orthopaedic therapies. The microenvironment the cells experience within a repair tissue will play a major role in the repair response. Within the musculoskeletal system, one of the major regulators is the mechanical load applied to the cells within the defect.

Most new cartilage repair therapies are developed using static culture. However, it is clear that due to the critical role mechanics plays in vivo, a more physiological loading regime in vitro would be more appropriate and this can be achieved by the use of bioreactors. Using a multiaxial load bioreactor system, we have been investigating the effect of mechanical stimulation on human MSC differentiation. Performing studies in the absence of growth factors, specifically Transforming growth factor β (TGFβ), allows the direct effect of the mechanical strain applied to be elucidated. Our bioreactor system allows for the application of shear, compression or a combination of both stimuli to establish the phenotypic changes induced within MSCs. As a model system, human bone marrow derived MSCs are embedded in a fibrin gel, which is then retained in a macroporous biodegradable polyurethane (PU) scaffold. Neither compression alone, nor shear alone can induce a change in MSC phenotype within this system. However, a combination of compression and shear is able to induce chondrogenic differentiation and this is due to increased endogenous expression of TGFβ from the loaded cells.

We have identified targets that are differentially regulated in TGFβ induced chondrogenic differentiation compared to mechanical induction, one of the most interesting being an increase in nitric oxide (NO) production during mechanical stimulation. Therefore, NO is potentially increased during rehabilitation after microfracture and this effect would not be observed during static chondrogenesis studies.

Session No.: S27-03 Invited Speaker

Direct Reprogramming of Human Corneal Epithelial Cells Using Six Transcription Factors

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Corneal epithelium plays a critical role in maintaining the cornea's transparency. The corneal epithelium is maintained by continuous turnover of the corneal epithelial cell(CEC)s, which are supplied by the corneal epithelial stem cells that reside in the periphery of cornea. Severe damage to cornea, such as caused by Stevens-Johnson syndrome, results in depletion of the stem cells, leading to abnormal maintenance of corneal epithelium and to severe reduction of the vision. Allotransplants of cornea fail to restore the deficiency due to immunorejection. Several techniques have been implemented to use autologous cell sources that substitute for corneal epithelium. One approach is to use cultivated oral mucosal epithelial sheet. Although it has provided promising results for physical stabilization of the eye surface, because of oral mucosal identity this transplantation causes superficial light-scattering and corneal neovascularization that reduce the vision. To overcome these issues, derivation of autologous CECs is required. Here we sought to identify master transcription factor(TF)s in CECs that will allow us to induce these cells through enforced differentiation from iPSC or direct reprogramming from skin fibroblasts. 11 days after the transduction of transcription factors, the morphology of skin fibroblasts dramatically changed and looked quite similar to the primary CECs expressing keratin 3 and keratin 12.

Session No.: S27-04

Microrna Signalling Modulates Mesenchymal Stem Cell Fate in Response to Substrate Stiffness

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Mechanical signals from biomaterial scaffolds can direct the fate of mesenchymal stem cells (MSCs), affecting their proliferation, migration and differentiation. The considerable potential of MSCs for therapeutic applications will thus remain untapped until we fully understand the signalling mechanisms that underlie the response of MSCs to biomaterial properties. MicroRNAs can regulate all aspects of MSC fate, as well as key genes involved in mechanotransduction (integrins, cytoskeletal proteins and Rho/Rac/Cdc42), making them ideal targets to enhance fate specification of MSCs in biomaterial composites. However, the role of microRNA signalling in regulating the response of MSCs to physical cues is not yet determined. We developed a model system of MSCs cultured on stiff (40kPa) or soft (0.2kPa) polyacrylamide gels as well as stiff gels in the presence of C3T, an inhibitor of RhoA. MSCs cultured under these conditions displayed consistent changes in morphology and cytoskeletal architecture and had decreased RhoA and increased Rac1 activity on soft gels or with C3T. Furthermore, MSCs cultured on stiff gels were biased towards osteogenesis whereas those cultured on soft gels or with C3T were biased towards adipogenesis. MicroRNA sequencing revealed significant differences in miRNA expression between the different conditions, the predicted targets of which were enriched for genes involved in cell morphology, assembly and organisation, movement and proliferation and the actin cytoskeleton, as well as signalling pathways including RhoA, Rac and Cdc42. qPCR was used to validate a subset of the miRNA candidates and measure the expression of their predicted target genes. In MSCs transfected with candidate miRNA mimics/antimiRs, changes in target gene expression were confirmed and differences in the osteo-adipogenic bias of MSCs on the different substrates was noted. This provides the first proof-of-concept that modulation of miRNA signalling can be used to alter MSC fate in response to a physical cue.

Session No.: S27-05

Autologous Adipose-derived Stem Cells Prolong Survival of Vascularized Composite Allotransplantation in a Miniature Swine Hind-limb Model

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Background: Our previous study demonstrated donor mesenchymal stem cells (MSCs) could prolong vascularized composite allotransplant (VCA) survival. However, recipient adipose tissue is easier to harvest than donor tissue for pre-conditioning MSCs. This study investigated the efficacy of recipient autologous adipose-derived stem cells (rASCs) for VCA survival. Methods: Heterotopic hind-limb transplantation from female donors to male recipients was performed in out-bred miniature swine. Group-I were untreated controls. Group-II obtained rASC infusions (given on days 0,+7,+14, and +21). Group-III obtained Tacrolimus (FK506, day 0~+28). Group-IV obtained irradiation (day -1), FK506 (day 0~+28), and rASC infusions (day 0,+1,+7,+14,+21). Tissue samples were biopsied, and flow cytometry was performed to quantify T cell populations. ELISAs were used to measure the levels of TGF-β, IL-10, and TNF-α. Sex-determining region of Y-chromosome (SRY) expression of donor tissue was detected by using polymerase chain reaction (PCR) and immunohistochemical staining. Results: Treatment with multiple injections of rASCs along with irradiation and FK506 resulted in statistical increases in allograft survival. The percentage of CD4+/CD25+/foxp3+ regulatory T-cells revealed a significant increase in the rASC-irradiation-FK506 group as compared to controls. Analysis of recipient peripheral blood revealed that TGF-βl was significantly increased in the rASC-irradiation-FK506 group. The immunohistochemical staining and PCR analysis showed recipient sex-determining region of Y-chromosome (SRY) gene expression existed in donor allo-tissues in the rASC-irradiaion-FK506 group. Conclusion: Recipient ASCs in addition to irradiation and transient immunosuppressant treatment could prolong allotransplant survival, modulate T-cell regulation and enhance recipient cells engraftment into the allotransplant tissues.

Session No.: S28-01 Keynote Speaker

Functional Recovery in Osteoarthritic Chondrocytes Through Hyaluronic Acid and Platelet-rich Plasma-inhibited Infrapatellar Fat Pad-adipocytes

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Background: Recent studies have evidenced that higher adiposity in infrapatellar fat pad (IFP) induce inflammatory phenotype in the knee-joint and thereby contribute to development and progression of osteoarthritis (OA). In particular, IFP adipocytes–derived inflammatory cytokines participate in pathologic events. Our previous research has already addressed the therapeutic efficacy of hyaluronic acid and platelet-rich plasma (HA+PRP), including the promotion of cartilage regeneration and the inhibition of inflammation. Current study aimed to explore remedial action of co-administered HA+PRP in osteoarthritic recovery via IFP-adipocytes inhibition. Hypothesis: HA+PRP repairs OA articular cartilage through inhibiting the release of adipokines from IFP-Adipocytes. Study design: Controlled laboratory study. Methods: IFP-adipocytes and articular chondrocytes were obtained from 10 OA patients. Effects of releasate containing cytokines and adipokines in IFP adipocyte-derived conditioned medium (IACM) on articular chondrocytes and IFP-adipocytes itself were evaluated. Therapeutic efficacy of exogenous HA+PRP was determined through their administration to co-cultured IFP-adipocytes and articular chondrocytes and further demonstrated in 3D arthritic neo-cartilage model. Results: IACM and IFP-adipocytes–induced microenvironment could induce de-differentiated and inflammatory phenotypes in articular chondrocytes. HA+PRP decreased inflammatory potential of IFP-adipocytes through profound inhibition of cytokines and adipokines. IACM-mediated reduced cartilaginous extracellular matrix (ECM) could also be recovered through HA+PRP in 3D arthritic neo-cartilage model. Conclusions: IFP-Adipocytes-derived releasates mediated inflammatory response de-differentiation in chondrocytes which was recovered through HA+PRP administration. Clinical Relevance: Our findings demonstrated that HA+PRP effectively diminished IFP-adipocytes-promoted inflammation in articular chondrocytes which indicates that IFP could be a potential therapeutic target for OA therapy. Keywords: adipocytes; infrapatellar fat pad (IFP); osteoarthritis (OA); hyaluronic acid (HA); platelet-rich plasma (PRP)

Session No.: S28-02 Invited Speaker

Application of Biodegradable Methoxy Poly (Ethylene Glycol)-b-Poly (Lactide-co-glycolide) Hydrogels for Intra-articular Injection of Knee

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The articular cartilage of knee is known to worn out due to aging, trauma and pathological dysfunction. The synovial space of knee is filled with synovial fluid which works as shock-absorption and lubrication. Cross-linked hyaluronic acid is usually injected to the synovial space of the patients bearing osteoarthritis (OA) to relief the pain in knee. The present study aimed to develop a biodegradable hydrogel to supplement the synovial fluid for the patients bearing OA. Methoxy poly(ethylene glycol)-b-poly (lactide-co-glycolide) (mPEG-PLGA) was synthesized by ring-opening polymerization. The viscoelastic properties of mPEG-PLGA hydrogel were determined by a rheometer (Discovery HR3, TA Instruments) The elastic moduli and loss moduli of the hydrogel subject to different oscillation frequencies and temperature were then recorded. The molecular weights of diblock copolymer mPEG-PLGA ranged from 1222 Da to 3013 Da as determined by NMR spectra, while GPC measurement gave number-average molecular weights from 1838 Da to 3026 Da. The molecular weights of the polymers were proportional to the feedings of monomers (lactide and glycolide) in the polymerization. In the body the synovial fluid displays a crossover of elastic modulus and loss modulus at 1 Hz of the oscillation frequency which fits to the movements of the knee for walking and running. mPEG-PLGA hydrogels showed similar crossover in a frequency range of 0.25 Hz while commercial-available hyaluronic acid of 1.5 million Da has higher crossover frequency of 3.9 Hz. It indicates that mPEG-PLGA hydrogel, used as intra-articular injection, is expected to benefit general walking while the commercial product of hyaluronic acid is designed for robust movement of knee. The hydrogel prepared by biodegradable mPEG-PLGA exhibit viscoelastic properties when it was subjected to oscillation (shear stress). The polymers and the hydrogel could be further modified to meet the viscoelastic properties of synovial supplement for the patient bearing OA.

Session No.: S28-03

Acellular Cartilage Matrixc Combined with Chitosan Cellulose Hydrogel for Cartilage Regeneration: Cellcompatibility and Gene Expression Analysis

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Cartilage defect is the most common cause of disability in the world. Recently, there are various studies try to use extracellular matrix (ECM) to develop scaffolds for cartilage repair. In the study, we try to developed an acellular cartilage matrix (ACM) contained hydrogel for cartilage regeneration. WST-1 and LAL assay were used to evaluate the cell-compatibility and endotoxin content, while real-time PCR was used to analysis the mRNA gene expression of encapsulated mesenchymal stem cell. Results showed that ACM contained hydrogel are biocompatible with chondrocyte and bone marrow mesenchymal stem cell, and the endotoxin content was much lower than the toxic endpoints established by FDA. Importantly, we found type II collagen, aggrecan and SOX-9 mRNA of MSC were up-regulated, while TGF-β, MMP-2, MMP-13 and TIMP-2 mRNA were down-regulated. in ACM contained hydrogel. Base on above results, we believe that ACM contained hydrogel would be promising biomaterial for future cartilage repair.

Session No.: S28-04

Chondrogenic Induction of Bone Marrow Stem Cell Using Chondrocyte Conditioned Medium

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Cell-based treatments of osteoarthritis and other cartilage defect requires ex vivo culture of human cells to obtain enough cell number for implantation. However, it is challenging to produce a high quality ex vivo cultured cells or to find the alternative cell sources for cartilage tissue engineering. In this study, we aim to optimize chondrocyte conditioned medium (CCM) and analyse its chondrogenic ability. BMSCs were subjected to chondrogenic induction using chondrocyte conditioned medium (CCM) and chondrocyte induction medium (CIM). Chondrogenic ability was determined based on CCM-induced BMSCs (iCCM) and CIM induced BMSCs (iCIM) gene expression. CCM condition was optimised based on immunocytochemical analysis, safranin O staining, and protein quantification analysis. In terms of morphology, iCCM starts to aggregate together, however there is no presence of clump compared to iCIM. For gene expression, CCM caused down regulation of collagen type 1 and collagen type X protein meanwhile CIM causes up regulation of ACP and SOX 9 gene. Immunocytochemical (ICC) analysis showed that, collagen type 2 expression decreased throughout the culture time for all tested groups. All tested groups showed positive staining of safranin-O as early as day 7. Protein quantification revealed that the highest amount of protein for CCM was obtained at passage 3 of chondrocyte culture at 72 hours collection time. As conclusion, 50:50 ratio of CCM to fresh medium using chondrocytes at passage 3 with 72 hours conditioning time were chosen based on iCCM morphology, protein marker, presence of proteoglycan and secreted protein concentration. Further study including secretome analysis will be done to validate the results and to predict the potential pathway, regulatory mechanism and mediators that leads to chondrogenic lineage.

Session No.: S28-05

Therapeutic Effects of Neuropeptide Substance P Coupled with Self-assembled Peptide Nanofibers on the Progression of Osteoarthritis

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Osteoarthritis (OA) is a progressively degenerative disease that is accompanied by articular cartilage deterioration, sclerosis of the underlying bone and ultimately joint destruction. Although therapeutic medicine and surgical treatment are done to alleviate the symptoms of OA, it is difficult to restore normal cartilage function. Mesenchymal stem cell (MSC) transplantation is one of the therapeutic trials for treating OA due to its potential, and many researchers have recently reported on the effects of MSCs associated with OA therapy. However, cell transplantation has limitations including low stem cell survival rates, limited stem cell sources and long-term ex vivo culturing. In this study, we evaluated the efficacy of neuropeptide substance P coupled with self-assembled peptide hydrogels in a rat knee model to prevent OA by mobilizing endogenous MSCs to the defect site. To assess the effect of the optimal concentration of SP, varying concentrations of bioactive peptides (substance P (SP) with self-assembled peptide (SAP)) were used to treat OA. As shown by our results, the SAP-SP hydrogel accelerated tissue regeneration by anti-inflammatory modulation shown by an anti-inflammation test using dot-blot in vitro. Additionally, the treatment of OA in the SAP-SP group showed markedly improved cartilage regeneration through the recruitment of MSCs. Thus, these cells could be infiltrating into the defect site for the regeneration of OA defects. In addition, from the behavioral studies on the rats, the number of rears significantly increased 2 and 4 weeks postinjection in all the groups. Our results show that bioactive peptides may have clinical potential for inhibiting the progression of OA as well as its treatment by recruiting autologous stem cells without cell transplantation.

Session No.: S28-06

Decellularized Cartilage Scaffold for Osteochondral Regeneration and Reconstruction

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Background: Decellularized tissues have been used successfully in reconstructive and regenerative medicine. Conventional decellularization involves multiple chemical processing steps to ensure completion of cell removal to minimize unwanted host tissue immune responses. To mitigate the inflammation risk of chemical decellularization, supercritical carbon dioxide (SCCO2) extraction technology was investigated for its effectiveness in cartilage tissue decellularization. Methods and Results: Porcine nasal cartilages were obtained from 6-month old pigs. The cartilaginous tissues were first incubated in NaOH and low concentration hydrogen peroxide, followed by SCCO2 extraction to complete tissue decellularization. The decellularized cartilage (DC) was evaluated histologically by hematoxylin-eosin (H&E), 4',6-diamidino-2-phenylindole (DAPI), and Alcian blue stains. The microstructures of DC were examined by scanning electron microscopy (SEM). Biocompatibility of DC was assessed in 3T3 cell cultures. Results of histology showed significant reduction in the number of cells and nuclei as well as decreased amount of non-collagen proteins, such as glycosaminoglycan (GAG). At the microstructural level, the SCCO2-processed tissues appeared similar to the unprocessed tissue. 3T3 cell viability was excellent as cells were able to migrate into the decellularized scaffold and proliferate in 7-day and 14-day cultures. Conclusions: The combination of mild chemical treatments and SCCO2 extraction were able to remove majority of cells from the cartilaginous scaffolds. The use of SCCO2 extraction in the terminal step has the additional advantage of eliminating chemical residues that remained in the prior process steps. SEM results showed no microstructural changes that could compromise biomechanical properties. The DC produced by this simple process can potentially be an ideal osteochondral scaffold for cartilage regeneration.

Session No.: S29-01 Keynote Speaker

State-of-the-art Technology for Cell Manufacturing

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Cell culture, which is the most critical steps to realize the transplant of cells or tissues for cell therapy or regenerative medicine, respectively, will be done in terms of safety and cost-saving under the aseptic environment in the specific cell processing facility (CPF) to earn the doctors and patients secures. The realization of 3S (safe, cost-saving and secure) is desired for the industrialization because the cost for cell processing and stability of the final products for therapy are not sufficient. Currently, cell processing isolators can enable cell processing in a closed aseptic environment, which may reduce equipment and maintenance/operation costs while providing a reliable aseptic environment. In case of autologous cell processing, the CPFs are expected to handle cells collected from a large number of patients, and some believe that isolators with a function to prevent cross-contamination may be advantageous in providing a more reliable aseptic environment compared with open operation in the facilities. In the present study, a novel isolator system based on a flexible Modular Platform (fMP) was designed to realize that the individual modules can connect and disconnect flexibly with keeping the aseptic environment in each module, applying the cell culturing. In addition, as it is known that the serial processes for cell processing affect the quality of the cells, the automation of the processes is conducted not only to maintain an aseptic environment but also to lead to stable processing in CPF. Thus, our attempts are concluded to build an advanced culture system employing isolator technology, and the adaptation of the fMP in CPF will lead to easy installation of the new modules for production line addition and/or revision through the clinical phases as well as commercial production, which contributes to the reduction of production costs.

Session No.: S29-02 Keynote Speaker

Global Efforts Towards Hta of Regenerative Medical Products for Patients

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Regenerative medical products have the potential to meet unmet medical needs and improve health outcomes. In Japan, Regenerative Medicine Promotion Act, Regenerative Medicine Safety Law and PMD Act are enacted and new regulatory category of regenerative medical product and expedited approval system of conditional, time-limited marketing authorization have been established since November 2014. Regulatory review issues for confirming safety and predicting efficacy have been examined under dialgue between academia and PMDA professionals. Four regenerative medical products have been approved with NHI insurance pricing under shorter review period and better understanding. Automation of living cell isolation and manipulation process together with its quality management guidelines are under development to realize affordability for responding patients. In contrast to conventional pharmaceutical products or medical devices, regenerative medical products are uniquely integrated which poses challenges when determining product value, certainty, reimbursement, guidelines for clinical use. The USA and EU have pricing systems based on review of outcome data, while Japan has cost-plus pricing system for innovative product without outcome database. In the UK, NICE and University of York have published their technology appraisal study of assumed CAR (chimeric antigen receptor) T-cell therapy specific to antigen CD19, for treating relapsed or refractory B-cell acute lymphoblastic leukaemia (B-ALL), following a recommendation from the UK Regenerative Medicine Expert Group. In the US and EU, newly designed payment scheme is being considered. In Japan, the Government has encouraged clinical researchers to investigate costs through clinical development. AMED, newly established funding agency, funded special survey in 2015 to discuss necessity of value-based pricing which may work well with improved certainty of patient remission under conditional, time-limited marketing authorization system. I will overview UK case study and our development efforts of health technology appraisal for regenerative medical product in Japan and raise importance of global collaboration to meet challenges.

Session No.: S29-03 Invited Speaker

Cell Therapy at the Crossroads: The Conquests and Constraints of the Current Time & the Way Forward!

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Cell therapy, GMP cell expansion in automated closed system

Session No.: S29-04 Invited Speaker

Market and Clinical Unmet Need Oriented Development of Regenerative Medicine Products: From Ideas to the Market

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Researches of the regenerative medicine technologies and products are only the beginning of the product development. After the proof of concept of the research ideas, market analysis for the commercialization process is the first step. Regulatroy issues determine the critical steps of product testings, including pre-clinical tests and possibly clinical evaluation. Then the business plan shall be created. The most important issue includes the business model and financial needs. We have started up an innovative platform focusing on design, development and pilot manufacturing for medical technologies. We set up special expertise on design and development of biomaterials, medical implants, cell therapy procedures, and innovative medical devices. By partnership with medical doctors and scientists, the clinical unmet needs are discovered and transformed to potential medical products. The objective is to maximize the values of the developed medical products based on the innovation, incubation, and integration processes.

Session No.: S29-05 Invited Speaker

Scientific Challenges for the Safety, Efficacy and Quality of Cell-based Therapeutic Products

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Development of regenerative/cellular therapies using cell-based therapeutic products (CTPs) is keenly anticipated for patients suffering from currently incurable diseases/damages. A lot of efforts have been, and are made to develop new legislations for translation of novel CTPs. For instance, the Japanese Diet recently passed the Regenerative Medicine Promotion Act, as well as the Pharmaceuticals and Medical Devices Act (the Revised Pharmaceutical Affairs Law) and the Regenerative Medicine Safety Act. The European Medicines Agency (EMA) has started the adaptive pathways approach, a new regulatory framework to improve timely access for patients to new medicines including CTPs. The U.S. House of Representatives passed the 21st Century Cures Act in July 2015, which would require FDA to issue guidance on accelerating innovative therapies, including regenerative medicine and genetic technologies for rare diseases. However, the development of CTPs is still uncertain, because they include advanced and emerging technologies with limited clinical experiences. One of the biggest problems is that evaluation tools and approaches to ensure their safety, efficacy and quality are often lacking, which affects not only the risk assessment/management of clinicians, industries and regulators, but also the risk communication with the public. This is one of the major reasons why regulatory/translational sciences are critical for CTPs. At the session, I would like to overview current scientific challenges for the development of CTPs, and introduce our studies to establish new methods for risk assessment of CTP-derived tumor formation, as an example of regulatory science researches.

Session No.: S29-06 Invited Speaker

Regulatory Perspectives of Japan

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In Japan, the regulatory reform was carried out to improve access to the new therapeutic innovation in regenerative medicine. The Pharmaceuticals and Medical Devices Act (PMD Act) (the revised Pharmaceutical Affairs Law) became effective on 25 November 2014, which enables early patient access to promising therapies, using conditional and time-limited approval scheme (as “accelerated approval”) for regenerative medical product review.

Under the new regulatory scheme, the two new products were approved in September 2015; one is MSC for GVHD second line therapy, the other is skeletal myoblast sheets for ischemic heart failure. The latter one was given conditional and time-limited authorization for 5 years.

For the evaluation and manufacturing control of human cell therapy products (hCTPs) that are different from traditional biological protein products, the some specific points to consider will be flexibly accommodated with respect to quality, safety and efficacy evaluation as presented in this discussion, though most of the conventional biologics regulations, including ICH guidelines are even appropriate. Also, such an early access scheme would raise the issues of GMP type quality validation, due to the limited experience of manufacturing batches and the frontloaded timeline in the regulatory process. Risk-based scientific evaluation should be applied to achieve patient protection.

I will present current PMDA’s regulatory perspectives to facilitate product development and to streamline review system for enabling regenerative medicine.

Session No.: S30-01 Keynote Speaker

Gelatin Nanoparticles with Tea Polyphenol Loading as Eye-drops for Eye Disease Treatment

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Eye-drops is the most common formulation for ocular drug delivery, but only 5 percent of the administered dose retained in eyes after 5 min. The role of the ophthalmology care is focused on increasing drug bioavailability in ocular tissue. In order to develop an effective carrier for ocular drug delivery, biodegradable polymer, gelatin, is used to prepare nanoparticles for drug encapsulation. With hyaluronic acid (HA) addition, interaction of nanocarriers and cornea can be increased by mucus adhesive improvement. There is two common eye diseases were focused for test eye-drops with nanomedicine in it, one was dry eye syndrome (DES), and the other was corneal neovascularization (NV). Epigallocatechin gallate (EGCG), the major tea polyphenols in green tea, was loaded in the gelatin nanoparticles for treating these two diseases due to its anti-inflamation and anti-angiogenesis capacity. A rabbit model with dry eye syndrome was established and tested for confirm the therapeutic effect of eye-drops with gelatin/EGCG/HA nanoparticles (GEH) in it. Mice eyes were alkaline burned to create vessels ingrowth in cornea, then treated by eye-drops contained GEH nanoparticles with RGD modification on its surface (GEH-RGD). Overall, we confirmed that eye-drops with nanoparticles in it could cause the longer and higher drug accumulation in the cornea, and then enhance the therapeutic effect in both DES and cornea NV animal model. DES rabbits treated by GEH nanoparticles show higher tear secretion and lower inflammatory signs; and corneal NV mice treated by GEH-RGD nanoparticles reveal less vessels formation and low VEGF/MMP content in cornea. According to these results, nanomedicine can be used as an eye-drop for the treatment of eye disease for clinics in a very easy way.

Session No.: S30-02 Invited Speaker

Cell-based Therapy for Treating Corneal Endothelial Dysfunction

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The corneal endothelium maintains corneal transparency via its pump and barrier functions. Consequently, its decompensation causes severe vision loss due to corneal haziness. Fuchs' endothelial corneal dystrophy and decompensation of corneal endothelium post cataract surgery are the leading causes of corneal endothelial dysfunction. At present, corneal transplantation is the only therapeutic option for treating corneal endothelial dysfunction. However, regenerative medicine now offers researchers an attractive alternative pathway towards providing innovative therapies. We previously demonstrated that the inhibition of Rho-associated protein kinase (ROCK) signaling by a specific ROCK inhibitor promoted cell adhesion to the cell culture substrate. In a later study, we applied ROCK inhibitor to modulate the cell adhesion properties of cultivated corneal endothelial cells (CECs) from the aspect of creating a cell-based therapy. In both rabbit and monkey corneal endothelial dysfunction models, we demonstrated that the transplantation of cultivated CECs in combination with ROCK inhibitor resulted in the successfully recovery of corneal transparency via reconstruction of the corneal endothelium. One difficult technical obstacle that we did encounter was that of culturing CECs, as they have poor proliferative ability and exhibit fibroblastic phenotypes with loss of pump and barrier functions while under the culture conditions. However, we successfully overcame that obstacle by developing a CEC culture protocol which enables an efficient in vitro expansion of the cells for clinical use. Of interest, we recently obtained approval from the Japanese Ministry of Health, Labour, and Welfare to treat corneal endothelial dysfunction via the cell-based therapy, and started the first-in-human clinical trial in 2014 at Kyoto Prefectural University of Medicine in Japan. In this presentation, the preliminary results of our cell-based therapy for the treatment of corneal endothelial dysfunction will be introduced.

Session No.: S30-03 Invited Speaker

Corneal Stromal Tissue Engineering Using Keratocyte Spheroids Fabricated on Chitosan Coatings with Different Deacetylation Degrees

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Corneal stroma reconstruction is an attractive research field in tissue engineering since it is of high clinical importance. The aim of this work was to investigate the effect of deacetylation degree (DD) of chitosan on the fabrication and application of keratocyte spheroids for regenerative medicine. By means of heat-alkaline treatment under a nitrogen atmosphere, the DD of as-received chitosan was significantly increased from 74.1 ± 0.5% to 94.2 ± 0.5% while maintaining similar molecular weight. After surface coating of tissue culture plates with chitosan of varied DDs, three test groups of substrate samples (C74, C84, and C94) were characterized by contact angle measurements, tensile tests, and X-ray diffraction. Our results showed that the surface wettability, mechanical strength, and crystallinity of chitosan coatings significantly increased with increasing DD. The rabbit corneal keratocytes (RCKs) on the C74 groups exhibited poor adhesion and spreading than did those of C84 and C94 groups. At the early stage of cultivation, the RCKs exposed to various chitosan materials began to form spheroidal aggregates. Additionally, the high percentage of larger-sized spheroids was noted on the samples with decreasing DD. An animal model of bacterial keratitis was further used for evaluation of therapeutic efficacy of RCK spheroids. Results of corneal thickness measurements and slit-lamp biomicroscopy showed that when compared to the isolated cell suspensions, the bioengineered multicellular spheroids were more beneficial to corneal stromal tissue reconstruction. At 14 days of post-injection, the rabbit cornea receiving larger-sized RCK spheroids presented better tissue repair. It is concluded that the DD of chitosan may play an important role in the fabrication of bioengineered multicellular spheroids for use in corneal stromal tissue engineering.

Session No.: S30-04 Invited Speaker

3D Spherical Culture and Bioreactors for Corneal Tissue Engineering

Jiansu Chen[1], Jun Zhang[1], Shanyi Li[1], Ying Dai[1], Yonglong Guo[1], Hongyang Li[1], Xiaoxia Li[1], Yuting Han[1], Jing Meng[1], Hongwei Deng[2]

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In order to construct corneal layers closer to the natural cornea in vitro, 3D spherical culture, simulate microgravity (SMG) and perfusion bioreactors for corneal epithelial cells (CEpC), stromal cells (CSC) and endothelial cells (CEnC) were investigated. 10μM Y-27632 and 0.3μM Y-39983 significantly enhanced the survival of injectable CEpC and CEnC spheroids, which was favorable to form CEpC and CEnC tissue engineering sheets. Comparison with single cells, CSC and CEnC spherical culture promoted the proliferation and expressions of stem markers of CD34 and Nestin by RT-PCR assay and immunofluorescence staining. Under SMG culture, robust growing CSC aggregates showed the gene and protein expressions of keratocan and lumican of keratocyte phenotype even in the presence of 10% FBS. CSC aggregates or sheets revealed the complex network of triangular or polygonal dendritic morphology of the cell bodies with many fine and long processes, which adhered to the scaffolds tightly in SMG condition. Adipose derived stem cells (ADSCs) obtained higher differential potential for CEnC commitment after direct reprogramming with PTD-Oct4/KLF4/Sox proteins and purmorphamine in SMG bioreactor. Perfusion culture was helpful to CEnC hexagonal shape, proliferation, ECM formation and barrier function. In summary, 3D spherical culture, SMG and perfusion bioreactors increase the viability, stemness and injectability of CEpC, CSC and CEnC of cornea, which are conducive to tissue engineering and 3D bioprinting cornea closer to the natural cornea.

Session No.: S30-05 Invited Speaker

Comparison of the Effects of Supercritical Carbon Dioxide-based Process and Surfactant-based Process on Porcine Cornea Xenotransplantation Potential

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INTRODUCTION: Chemical and enzymatic methods have been used to remove endogenous cellular components from xenografts. Unfortunately, these methods can introduce process residues that can cause bioincompatibility and increase the likelihood of graft rejection. A more benign method is needed to produce biocompatible xenograft corneas. Application of supercritical carbon dioxide (scCO2) fluid in tissue de-cellularization has been reported. Here we compare porcine corneas decellularized using the scCO2 process to corneas decellularized using Triton X-100.
METHODS: Corneas were harvested from porcine eye balls and soaked sequentially in reverse osmosis water and sodium chloride (NaCl) solution. Finally, NaCl solution-treated corneas were subjected to scCO2 extraction in a high-pressure reaction apparatus (Applied Separations Inc.) to complete the de-cellularization procedure. For the Triton X-100 procedure, corneas were incubated in 0.2% Triton X-100 for 6 hours after NaCl treatment. Decellularization was assessed by histology. Decellularized corneas were tested for tissue porosity, absorbency, and tensile strength. Biocompatibility was assessed in cell cultures and in animal studies. Safety and performance were assessed in a rabbit cornea transplantation model.
DISCUSSION & CONCLUSIONS: Both methods successfully removed endogenous corneal cells, as verified by histology. In terms of physical characteristics, scCO2–processed corneas had greater absorbency (25.22±4.11 vs. 14.33±3.45 g/100m2; $p<0.05$) but lower tensile strength (stress max. load: 0.51 vs. 0.95 MPa) in comparison to Triton X-100-treated tissues. In vitro cell growth assay showed better cell attachment and proliferation on scCO2-processed scaffolds. When tested for bioburden, no microbial contamination was found on scCO2-processed corneas while Triton X-100-processed corneas were contaminated. Terminal sterilization by gamma irradiation had no effect on cornea absorbency regardless of the process methods. With the exception of material tensile strength, scCO2-processed corneas were equal or superior to Triton X-100-treated corneas in all areas of measurement. ScCO2 is a promising tissue processing technology worth further exploration.

Session No.: S31-01 Keynote Speaker

The Construction and Translational Research of Vascularization and Neurotization of Tissue-engineered Bone and the Mechanism of Osteogenesis Research

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The treatment of large bone defects still remain as a major clinical challenge worldwide and the bone tissue engineering provides a promising solution. We have conducted extensive and intensive research focusing on the construction and translational research of vascularization and neurotization of tissue-engineered bone. We have achieved several scientific discoveries and technological breakthroughs. (1) Highly diversified MSC sources from representative tissue origins have been meticulously and systematically screened and it has been demonstrated that MSC derived from bone marrow were the optimal cellular sources for BTE application. Systematic investigation has been carried out in different species, from small animals (mice, rat, rabbit), large animal (goat) to non-human primate (monkey) and human being to study the MSC behavior and its characteristics. Moreover, large scale MSC expansion techniques and a novel bioreactor system for clinical use have been established and successfully commercialized. (2) We are the first in the world to propose the synergetic approach of pre-vascularization and pre-innervation strategies in BTE research and demonstrated the efficacy and superiority of this synergetic strategy in preclinical large animal and non-human primate animal models. We have demonstrated that the vascular bundle implantation could achieved similar enhancing outcome in terms of innervation and bone defect healing. We also have demonstrated that independent peptidergic innervation can exist in bone tissue before the angiogenesis, and the CGRP (calcitonin gene related peptide) were the key factor for the osteogenetic mechanism of vascularization and neurotization. (3) We developed the standard operation procedures for the preparation of MSC and its clinical application and established the clinical surgery and evaluation protocols focusing on the clinical use scenario. We successfully conducted the first clinical trial to repair a 12cm critical-size tibial bone defect using the tissue engineered bone. Nine months after the operation, X-ray and CT examination showed fracture line

Session No.: S31-02 Keynote Speaker

Development of Bioactive Small Diameter Vascular Grafts

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Vascular disease, particularly coronary artery occlusion, is one of the leading causes of global deaths. The number of coronary artery interventions is projected to increase considerably owing to the rapid growth of the ageing population. However, one challenge is the limited availability of autologous vessels, which has lead to an increasing demand for vascular prosthesis suitable for coronary and peripheral vascular procedures. In the past decade, our lab has been focusing on development of small diameter vascular graft with synthetic materials. We realize that the ideal vascular graft should meet three conditions: rapid endothelialization; regeneration of vascular smooth muscle cells layer with contraction and relaxation property, and long-term patency with vascular homeostasis. Our aim is to regenerate the vascular grafts into neoarteries in vivo which are identical to the native ones in terms of structure and function. We devote in two aspects: 1) to optimize the structure of the scaffolds, we fabricated fibrous, macro-porous, elastic and biodegradable scaffolds by electrospinning, wet spinning, melt spinning and phase separation techniques; 2) to enhance the biological activity, we incorporated functional peptides, transcription factors, cytokines, polysaccharides etc, into the vascular grafts by chemical grafting or physical encapsulation methods. We evaluated these grafts by implantation in rat abdominal aorta and rabbit carotid artery. Through these approaches, we developed several kinds of vascular grafts which showed rapid endothelialization, enhanced cell infiltration, smooth muscle layer regeneration and ECM secretion. Moreover, we recently developed new functional grafts with controlled release of nitric oxide, or incorporated peptides that demonstrated excellent bioactivity in inducing the regeneration of neoarteries. References: 1. Wang Z, et al., J Control Release. 2015;210:179-188. 2. Zhu M, et al., Biomaterials. 2015;61:85-94. 3. Wang Z, et al., Biomaterials, 2014;35:5700-10. 4. Yao Y, et al., Acta Biomater. 2014;10:2739-49. 5. Zhu M, et al., Acta Biomater. 2014;10:2014-23.

Session No.: S31-03 Invited Speaker

Mesenchymal Stem Cell-derived Microvesicle Functionalized Decalcified Bone Matrix Scaffolds with Enhanced Pro-bone Regeneration Activity

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Vascularization is crucial for bone regeneration in the transplantation of tissue engineered bone grafts in clinical applications. Growing evidence suggests that mesenchymal stem cell-derived microvesicles (MSC-MVs) possess strong pro-angiogenic ability in vitro and in vivo. In this study, we fabricated a novel MSC-MV functionalized scaffold with enhanced pro-bone regeneration activity by coating MSC-MVs onto a decalcified bone matrix (DBM). The pro-angiogenic potential of MVs released from rat bone morrow derived MSCs was first investigated in vitro. In culture of human umbilical vein endothelial cells, cell proliferation, migration and tube formation abilities were enhanced in the presence of MSC-MVs. MSC-MVs were then coated onto DBM scaffolds and modification with MVs was demonstrated by scanning electron microscopy and confocal microscopy. The pro-bone regeneration ability of MV-modified scaffolds was then evaluated in a subcutaneous bone formation model in nude mice. After 1 and 2 months of implantation of scaffolds with or without seeding cells (osteogenic-induced rat bone marrow MSCs), bone formation evaluated by micro-computed tomography scanning analysis showed that MV-modified scaffolds enhanced bone formation, especially in the central region of the grafts. Enhanced bone formation was further confirmed by histological analysis. Immunohistochemical staining of CD31 proved that MV-modified scaffolds promoted vascularization in the grafts, indicating that the MV-modified scaffolds probably enhanced bone regeneration by accelerating vascularization. This novel scaffold modification approach provides a promising way to promote vascularization and bone regeneration which is crucial for bone tissue engineering.

Session No.: S31-04

A VEGF-binding Heparan Sulphate That Re-establishes Blood Flow in Murine Hindlimb Ischaemia

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Peripheral arterial disease affects approximately 12% of the adult population worldwide. Current standard-of-care involves lifestyle changes, medication or surgical interventions. However, long-term medication is still required to minimise the risk of recurrence. Emerging cell- or molecular-based treatment strategies aim to modulate the levels of vascular endothelial growth factor-165 (VEGF165), a potent blood vessel-forming factor. However, these strategies have not been approved for clinical use due to significant side effects. Using a heparan sulphate tuned to sequester endogenous VEGF (referred to as HS7, Wang et al., 2014), we investigated the ability of HS7 to promote endogenous recovery in a murine hindlimb vascular ischaemia model. In our current study, animals received daily intra-muscular injections of the respective treatments and were monitored using magnetic resonance angiography, laser Doppler imaging and limb function assessments. Blood volume in the ischaemic limb showed a dose-dependent recovery in animals receiving HS7 compared to control post-treatment. The blood volume in animals receiving the largest dose of HS7 was 5-fold higher than control at 8 days. In addition, the rate of recovery in blood volume over the first 8 days post-injury was greatest in animals treated with HS7 compared with control: Pre-surgery blood volume levels were recovered by ~6 days in HS7-treatment animals compared to control (~9 days). Laser Doppler imaging of the footpad showed blood perfusion to be ~1.3-1.5 times higher in animals receiving HS7 compared to control. Notably, 25% of HS7-treated animals had fully recovered limb function by 7 days, a number that grew to 91% and 97% at 14 and 21 days, respectively. In comparison, control only recovered to 81% by day 21. These results demonstrate the clinical potential of glycosaminoglycans like HS7 for application at sites of vascular insult and stress the importance of harnessing the activity of pro-healing factors generated at injury sites.

Session No.: S31-05

Nitric Oxide Promotes Endothelial Differentiation of Mesenchymal Stem Cells and Enhances in Vitro and in Vivo Angiogenesis

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Improving the pro-angiogenic properties of adult stem cells may contribute to their ability to enhance wound healing and also vascularisation of tissue engineered constructs. Nitric oxide (NO) is a diffusible gas with many pleiotropic properties including angiogenesis and in endothelial cells it is produced by endothelial nitric oxide synthase. In this study rat bone marrow derived mesenchymal stem cells were genetically modified by lentiviral vectors to enhance NO production. Angiogenesis was assessed in vitro and also in vivo by implanting subcutaneously a polyurethane scaffold containing modified cells within a fibrinogen gel. Activation of eNOS is controlled through a tight association with a scaffolding protein cavolin-1 (CAV-1). This eNOS-CAV-1 interaction inhibits NO production. rBMSCs were genetically modified to create a non-inhibitory eNOS-CAV-1 interaction by co-expressing eNOS and a mutated caveolin-1 (CAV-1F92A). Co-expression of eNOS and CAV-1F92A in rBMSCs significantly increased NO production compared to eNOS alone and increased in vitro capillary tubule formation and migration of cells in an in vitro wound healing assay both of which were reduced with the specific nitric oxide synthase inhibitor L-NG-Nitroarginine Methyl Ester (L-NAME). Gene expression analysis showed up regulation of the endothelial-specific markers CD31 (6.4 ± 0.42 fold) and V-cadherin (9.8 ± 2.02 fold) and increased expression of the pro-angiogenic genes VEGF-A, PDGFRα, FGF2, and FGFR2 in rBMSCeNOS+CAV-1F92A compared to cells expressing eNOS alone and controls. In vivo angiogenesis was assessed following subcutaneous implantation of rBMSCeNOS+CAV-1F92A and control cells seeded within a novel fibrinogen-polyurethane scaffold in the dorsum of rats. Implanted cells with increased NO production showed evidence of differentiation into endothelial cells through increased expression of CD31 and lectin and in vivo blood vessel formation. In summary, manipulation of NO levels may improve cellular reprogramming of adult stem cells towards an endothelial linage and may provide a novel

Session No.: S32-01 Keynote Speaker

Delivery of MiRNA Therapeutics and Anabolic Genes for Bone and Cartilage Engineering

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Delayed union/non-union resulting from bone fractures or serious trauma remains a challenging problem for orthopaedic surgeons. Conversely, cartilage defect or degeneration due to trauma or joint diseases can lead to debilitating pain and physical impairment. These problems have inspired the development of tissue engineering, which combines cells, biomaterials and biological signals, to stimulate cartilage/bone regeneration. Over the past decade, gene therapy has converged with cartilage and bone engineering, by which an increasing number of therapeutic genes are explored to stimulate cartilage and bone repair. These genes can be administered to cells via in vivo or ex vivo approaches using either viral or nonviral vectors. This presentation will focus on the use of viral vectors for genetic engineering of mesenchymal stem cells for cartilage/bone regeneration. In particular, emphasis is placed on the applications of baculovirus, an emerging nonpathogenic gene delivery vector, for the repair of cartilage and bone.

Session No.: S32-02 Invited Speaker

Biopharmaceutics-based Pancreatic Islet Cell Implantation for Diabetes Therapy

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Pancreatic islet transplantation is the ideal therapy for the treatment of type 1 diabetes mellitus (T1DM). However, transplanted islet grafts suffer oxidative stress and nonspecific inflammatory reactions. In addition, the damaged islets release a high-mobility group box 1 (HMGB1) protein, a cytokine-like transcription factor, which can eventually induce inflammatory responses via TLR (Toll-like receptor)-2, TLR-4, and RAGE (receptor for advanced glycation end product) pathway. Therefore, we focused on natural molecules such as bilirubin and glycyrrhizin for successful islet implantation. Glycyrrhizin, a glycoconju-gated triterpene produced by the licorice plant, can act as an inhibitor against active HMGB1 by directly binding. Bilirubin has showed anti-oxidative and anti-inflammatory functions. In this study, we chemically and physically modified these molecules to be used as biopharmaceutics. Then we evaluated their therapeutic effect to xenogenically-transplanted islets in diabetic animal. Collectively, these results demonstrated that these natural molecules-based treatment would be a novel remedy for the successful outcome of islet xenotransplantation.

Session No.: S32-03 Invited Speaker

Magnetic Nanomaterials-based Gene Delivery for Stem Cell Engineering

Jeff Chien-Wen Chang[1], Rih-Yang Huang[1], Yee-Hsien Lin[1]

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A redox-sensitive polymer/metal nanocomplex system (PSPIO) for magnet resonance imaging (MRI) and efficient magnetofection on cancer cells was successfully developed. SPIO exhibits favorable properties for gene delivery, such as: protect nucleic acids from enzymatic degradation, efficiently condense plasmid DNA (pDNA) into nanoparticles, exhibit redox-responsive pDNA release and maintain stable colloids in serum. Magnetically-assisted gene delivery enhancement on cancer cells was demonstrated from PSPIO in the presence of serum. PSPIO displayed good magnetization (28.3 emu/g) and dose-dependent T2-weighted imaging contrast (R2 = 291.1 s−1 mM−1) in vitro. In addition to cancer gene delivery, we are also interested in developing non-viral delivery vectors for stem cell engineering. Mesenchymal stem cells (MSCs) have emerged as a novel drug delivery platform due to their strong tumor tropism and biocompatibility. For example, MSCs expressed tumor necrosis factor-related apoptosis inducing ligand (TRAIL) has shown strong anti-cancer therapeutic efficacy. Due to their effectiveness, viral gene delivery is widely used to construct genetically engineered MSCs. Meanwhile, it is noticed that viral gene delivery methods should be used with caution for their clinical safety concerns such as insertion mutagenesis and induction of inflammatory responses. Alternatively, we proposed a nanomaterial-based gene delivery method (PNT method) to construct TRAIL-expressed human MSCs (hTRAILhMSCs). We have completed the evaluation of cytotoxicity of nanomaterials, human TRAIL expression from nanomaterials-transfected hMSCs, and in vivo anti-cancer cell effect of hTRAILhMSCs. PNT system was also attempted to deliver SOX9 or TGF-beta gene to hMSCs to promote chondrogenic differentiation. Significant chondrogenic differentiation enhancement (increased glycosaminoglycans (GAGs) and collagen II expression) was observed from hMSCs pellet culture using biochemical staining, immune-histological staining and real-time PCR analysis.

Session No.: S32-04

Virus Engineering for Cardiovascular Regeneration

So Young Yoo[1]

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Heart disease remains a leading cause of death worldwide. Due to the limited regenerative capacity of heart tissue, development of adequate cardiac regenerative therapy has emerged as an attractive approach. Recent work in cellular reprogramming of fibroblast into cardiomyocyte has shown important implications in potential regeneration therapies for cardiovascular diseases. However, retrovirus mediated transgene delivery in the system is not feasible in future applications to humans. In my presentation, virus engineering, which exploit the unique biological advantages from different type of viruses will be introduced and how these engineered viruses can be utilized for cardiovascular regenerating materials and therapeutic agent delivery. Based on recent clinical trials using recombinant virus mediated gene delivery, offering the promise that heart failure can be reversed via the system, I will discuss engineered virus AAV and/or M13 -mediated cardiovascular regeneration approach using mouse myocardial infarction model, which may facilitate future medical applications in regenerative heart medicine. [This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government(NRF-2014S1A2A2027641)]

Session No.: S32-05

Bioreducible Fluorinated Peptide Dendrimers as Efficient and Safe Gene Delivery Vehicles for Cancer Therapy

Xiaojun Cai[1], Zhongwei Gu[1]

[1]Sichuan University

Bioreducible Fluorinated Peptide Dendrimers as Efficient and Safe Gene Delivery Vehicles for Cancer Therapy Xiaojun Cai, Zhongwei Gu* National Engineering Research Center for Biomaterials, Sichuan University, Chengdu 610064, China xjcai@scu.edu.cn; zwgu@scu.edu.cn Despite showed great promise in the development of efficient and safe gene delivery system, the in vivo therapeutic efficiency of the existing polymeric vectors is still far from the requirements of clinical settings. This is mainly attributed to their poor performance on overcoming the multiple physiological and biological barriers. To address this critical issue and promote the clinical translocation of polymeric vectors, a new type of polymeric vector, bioreducible fluorinated peptide dendrimers (BFPDs), was rationally designed and synthesized by reversible cross-linking of fluorinated low generation peptide dendrimers. In virtue of masterly integrated all of the unique features of reversible cross-linking, fluorination and peptide dendrimers, this novel vector capable of effectively circumvent the entire extracellular and intracellular barriers for highly efficient and safe DNA and siRNA delivery, including unwanted disassembly in physiological milieu and attack by endogenous nucleases during circulation, limited cellular internalization, endosomal entrapment, inefficient polyplexes disassembly and cargo release in the cytoplasm. As a result, BFPDs showed excellent gene transfection efficiency in several cell lines and superior VEGF gene silencing efficiency (~65%) as well as strong capability to inhibit HeLa cell proliferation. Meanwhile, BFPDs not only revealed superior performance on long-term enrichment and retention in tumor sites after intratumoral injection, but offered considerable in vivo gene transfection efficiency and highly efficient tumor growth inhibition efficiency. More importantly, BFPDs not only revealed excellent in vivo anti-tumor efficiency but superior biocompatibility compared with Lipofectamine2000. This results therefore clearly verifies that BFPDs is a new class of efficient and safe delivery vehicles and should have remarkable potentials in clinical settings.

Session No.: S33-01 Keynote Speaker

Repairing Damage in the Nervous Tissue with Biomaterials and Neural Stem Cells

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Abstract: Potential: Neural stem cells (NSCs) can differentiate into neurons and glia, which provides a potential therapeutic application for many neurodegenerative diseases and neuronal injury. Biomaterials can form a network within the lesioned area, which provides physical support for the transplanted NSCs and bridges axonal regeneration. It is rational to combine both NSCs and biomaterials to repair the damage of CNS injury. Application: NSCs have been used for repairing nerve tissue damage in both animal models and patients. Basic research studies show that NSCs can survive, proliferate and differentiate into neurons and glial cells in injured site. NSC derived neurons can further make connection with neurons and seem to improve functional recovery. Biomaterials can enhance the survival rate and favor neuronal differentiation of NSCs both in vitro and in vivo. In clinical, NSCs have been used for treating nerve damage in patients. Problems remain: Although there have been many animal and clinical studies using NSCs for the treatment of CNS injury, several unsolved issues need to be addressed before cell transplantation can be considered for the clinical application. These issues include (i) choosing the right type and source of cells for transplantation; (ii) Using a right route and method for cell transplantation; (iii) improving the long-term survival of transplanted cells in the targeted area; (iv) enhancing the integration of transplanted cells with the host nervous tissue and make a right connection with the target; (v) improving host micro-environment for transplanted cells; (vi) understanding mechanisms of effect of cell transplantation for CNS injury. Conclusion: Cell transplant therapy combined with biomaterials holds a great potential for the treatment of CNS injury. Although there are unsolved issues need to be addressed, cell replacement represents one of the most promising therapeutic approaches for CNS injury such as in spinal cord injury.

Session No.: S33-02 Invited Speaker

Direct Conversion of Human Fibroblasts into Oligodendrocytes

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Spinal Cord Injury (SCI) involves apoptosis of resident oligodendrocytes (OLs), leading to progressive demyelination of neuro-pathways that could potentially be saved through remyelination of spared axons. Direct Conversion through Trans-differentiation is a safer and relatively faster cellular reprogramming approach that induces a cell lineage transition without passing through the pluripotent stages. Here, we report encouraging preliminary results for Direct Conversion of human fibroblasts to Oligodendrocyte Precursors (OPCs) by using a combination of three transcription factors (Sox2, Sox10, Olig2). This direct lineage conversion is induced through mRNA transfection, which is a safe and non-genome integrating transfection technique. In our ongoing research, we applied transient mRNA-GFP expression for ~5 days with ~90% transfection efficiency. The converted cells are positive for O4 (immature OPC markers) and Oilg1 and CNPase (late OPC markers), which indicates successful acquisition of early cells of the oligodendrocyte lineage. These converted cells could be expanded stately for three months and their survival, integration and maturation (expression of myelin basic protein (MBP) and myelin associated glycoprotein (MAG)) was validated in an in vivo shiverer mouse model. The neonatally engrafted converted OPCs, as traced by human nuclear antibody (HNA) could migrate and robustly myelinate the brains of myelin-deficient shiverer mice, and substantially decrease the shiver of these mice. Importantly, the converted OPCs were procured 4 weeks' post-transfection. This is especially significant for time-sensitive pathologies such as SCI. To further optimise the direct conversion process to attain higher population of converted and committed OPCs in a shorter period of time, we use piggybac transposon system for transient delivery of transcription factors through a 'footprint free' approach. A safe, fast and efficient conversion of autologous human cells to OPCs will open a new paradigm in human stem cell replacement therapy for patients with SCI.

Session No.: S33-03 Invited Speaker

Enhanced Dopaminergic Neuronal Differentiation on Topographical Pattern

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Microenvironmental topographies have been shown to influence the differentiation of stem cells. Appropriate biophysical cues can direct stem cell fate but the role of topography in differentiating stem cells into subtype specific cells has hitherto not been well understood. Parkinson's disease is a neurodegenerative disease attributed to the loss of midbrain dopaminergic (DA) neurons. Differentiation of stem cells into subtype specific cells may be guided by appropriate topographical cues but the role of topography has hitherto not been well understood. We aim to develop an in vitro substrate that will accelerate the derivation of midbrain dopaminergic neurons from human pluripotent stem cells (hPSCs) and neural progenitor cells. We first studied the neuronal differentiation of murine hippocampal neural progenitor cells (mNPCs) on a Multi-Architecture (MARC) chip with various topographical structures to identify the topographies that generated the highest percentage of neuronal (b-III-tubulin positive) and dopaminergic (tyrosine hydroxylase positive) populations. Subsequently, we examined the ability of topographical patterns to influence the differentiation of hPSCs into subtype-specific and regionalized dopaminergic neurons. We have made minor modifications to the protocol based on dual SMAD inhibition method and optimized on patterned substrates to further improve the efficiency for dopaminergic neuron derivation. From both type of stem cells, the topographical patterns could enhance the dopaminergic neuron differentiation. These results show the use of topographical influence for neuronal subtype specification, which could be translated into new therapeutic approaches for this neurodegenerative disease.

Session No.: S33-04 Invited Speaker

Neural Differentiation of Adipose-derived Stem Cells and Anti-inflammations Strategies for Peripheral Nerve Regeneration

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Adipose-derived stem cells (ASCs), an alternative source of adult mesenchymal stromal cells, possess high plasticity and can differentiate into neuronal lineage cells (NLCs) by using chitosan coated surface. Our recent studies discovered the therapeutic potential of human ASCs for sciatic nerve injury. We found the application of NLCs with chitosan coated nerve conduit significantly promoted the nerve regeneration with improvements of myelinated axons density and myelin thickness, gastrocnemius muscle weight and muscle fiber diameter, and gait functions. The intra-neural scarring tissue with high expressions of interleukin-1beta and leukotriene receptor 1 after nerve injury was inhibited when connected the nerve gap with chitosan-coated conduit and filled with NLCs. Since the anti-inflammation mechanism play important roles during nerve regeneration, we further tested the potential drugs that can be applied into the therapeutic microenvironments during surgery. Several compounds target to the inflammatory signals can be used in nerve conduit to modulate the dynamic inflammation process and achieved good regeneration outcomes. Therefore, the correct cell source and microenvironmental signals can benefit peripheral nerve regeneration after injury.

Session No.: S33-05 Invited Speaker

Niche Mimicking for Induction of Neural Stem/Progenitor Cells by Multilayer Films Based System

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The regulation of neural stem/progenitor cell (NSPC) niches, the guidance of neurite outgrowth, the induction of functional neurons, and the formation of functional synapses are the key components in the development of neural engineering. Polyelectrolyte multilayer (PEM) films have been used on cell behaviors regulation because of their capability of modulation of surface charges, thickness, and stiffness. Herein, a niche-modulated system by the formation of PEM films was fabricated to investigate surface effects on NSPCs differentiation. Then, a biomimetic system of supported lipid bilayer (SLB) with adsorbed sequential PEM films was adjusted to mimic the nature environment due to their structural similarity to synaptic membranes. Finally, a microfluidic system consisted of parallel indium tin oxide (ITO) surfaces in each chamber with and without PEM films was developed to compare the electrical field effect and surface properties of PEM effect. All of the systems in this study are serum- and growth factor -free system which provided a clean and clear platform to observe the interaction between surface niche and NSPCs differentiation. Neurite outgrowth length, differentiation lineage, and the synapse functionality were determined and compared to facilitate the suitable niches on neuron differentiation.

Session No.: S33-06

Scaffold-mediated Rna Interference in Directing Human Neural Stem Cells Differentiation

William Ong[1], Sing Yian Chew[1]

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Stem cell-based therapy offers tremendous potential in the field of regenerative medicine. The discovery of induced-pluripotent stem cells (iPSC) in 2006 opens a promising avenue to replacement therapy. The induction of iPSC-derived Neural Stem Cells (iPSC-NSC) into subtype specific neurons could be a potential treatment to many central nervous system diseases. However, bolus supplementation of soluble signals in the form of growth factors or cytokines has poor lineage selection and subtype specificity. It is known that biophysical and topographical cues could modulate stem cells fate; while small-interfering ribonucleic acid (siRNA) could silence repressive molecular pathways against desired lineage commitment. In our previous studies, we have shown that when coupled with topographical cues, we were able to enhance murine neural stem cells commitment to neuronal lineage. Recently, work has shown that topographical patterns are able to direct differentiation of murine neural stem cells into dopaminergic neuronal subtype. However, species difference between murine and human stem cells could be a hurdle in translating stem cell therapy into clinical use, and human iPSC holds an additional advantage over murine stem cells in its potential to provide personalized medicine. In this present study, we ask if the combination of topographical cues of nanofibers and gene silencing through siRNA could promote similar neuronal lineage commitment of iPSC-NSC just like their murine counterpart. RE-1 Silencing Transcription Factor (REST) siRNA is delivered through nanofibrous scaffold to achieve sustained gene knockdown in the iPSC-NSC to promote its neuronal lineage commitment. We take a step further by amalgamating molecular cues from soluble signals that are known to promote subtype-specific motor neuronal differentiation. Overall, we show by a multiple prongs approach, we are able to promote lineage and subtype-specific differentiation of iPSC-NSC.

Session No.: S34-01 Keynote Speaker

Design of Adhesive Growth Factors

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Growth factors are one of the main components for tissue engineering. They have been used as controlled release from matrices or immobilized state on matrices. They are used as they or after modification. The modification is regulation of affinity to matrices. If the affinity is low, the growth factors are released. If high, immobilization state is kept for a long time or targeting to a specific site is realized as targeting of antibodies. To add binding affinity to the growth factors, we devised some strategies. The first is usage of natural binding domains which are working in the bodies. By conjugation of matrix-binding domain, which was found in natural proteins, with growth factor, we developed some collagen binding growth factors. The second is utilization of in vitro selection method. Using the method, we can artificial develop various binding growth factors. We developed titanium or collagen-specific binding growth factors. By choosing the target, any binding growth factors are theoretically developed. The third is the bioorthogonal method inspired by underwater adhesion proteins. Since the active sites of adhesion proteins are made by post-translational modification, they contain non-canonical amino acids in the sites. We developed growth factors adhering various materials by incorporation of the non-canonical amino acids. Here the recent progress of these strategies for development of adhesive growth factors will be reviewed.

Session No.: S34-02 Keynote Speaker

Nanobiomaterials Potentiate Stem Cell-mediated Tissue Regeneration

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Nanobiomaterials can direct stem-cell fate both in vitro and in vivo by displaying stem-cell-regulatory signals in a precise fashion. Graphene and its derivatives can promote adsorption of cell-adhesion signals and soluble signals, which can be applied to enhancement of differentiation of mesenchymal stem cells (MSCs) into chondrogenic lineages. Nanobiomaterials can provide co-culture platforms for the generation of lineage-specific cells differentiated from MSCs that exceed the therapeutic potentials of non-modified MSCs. Graphene and its derivatives can serve as a cellular adhesive to prevent reactive oxygen species-mediated death of MSCs implanted to ischemia-damaged and reperfused myocardium and also can potentiate the myocardial repair efficacy of MSCs through stimulating expression of angiogenic growth factors and gap junction protein.

Session No.: S34-06 Invited Speaker

Adipose Tissue Engineering and Regeneration

Chen-Hsiang Kuan[1]

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Soft tissue defects resulting in deformity or asymmetry in appearance are difficult challenges to plastic surgeons. The defects are often resulted from trauma, post-tumor resection or congenital anomalies. People with contour abnormality usually lack of confidence and have lasting influences on their lives. Autogenous fat transplantation has the advantages of high tolerance. However, the non-lasting corrections and high absorption rates are the main concerns. In recent years, allogenic and natural materials have been developed and use as fillers for soft tissue defects. Nevertheless, their usage is frequently limited by foreign-body reaction, degradation over time, and etc. Adipose tissue engineering is an emerging field focuses on promoting regeneration and restore functions. The main challenges of developing adipose tissue are maintaining microvascular perfusion and preserving extracellular matrix (ECM). Natural and synthetic polymers are applied with much progress in soft tissue engineering. Advantages and disadvantages with respect to material mechanical and chemical properties, biocompatibility, and degradability would be introduced in this presentation. Current research strategies as cell therapy by applying adipose-derived stem cells shows great potential and would also be discussed here.

Session No.: S34-03

Inflammatory Regulation with Branched Peg for Tissue-engineered Vascular Graft

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Tissue-engineered vascular graft is expected to be used for substituting a host vascular tissue in vivo. In the previous study, we have developed a small-diameter long-bypass decellularized vascular graft by using ostrich carotid artery. In general, glutaraldehyde (GA) is used as the xenograft crosslinking agent for suppression of acute inflammatory response. However, GA-crosslinked tissue is not digested by an enzyme, and the tissue remains in long-term period. It is widely accepted that GA-crosslinked tissue has the risk to induce a calcification. Therefore, the GA is not able to be used for the suppression of inflammatory response in tissue-engineered graft. In this study, we study a surface modifier for decellularized vascular graft. Branched polyethylene glycols (PEG) activated with succinimidyl ester were employed. When the tissue was modified with the PEG, the tissue was gradually degraded in a collagenase solution (0.75mg/ml) although the unmodified tissue was rapidly decomposed. On the other hand, the GA-crosslinked tissue was not degraded under the same condition. When the PEG-modified tissue were transplanted into subcutaneous tissue of rats, infiltrated CD68-postive cells were not observed after 4 week transplantation. This tendency was same as the GA-crosslinked tissue. On the other hand, calcification was not observed on the PEG-modified tissue although the GA-crosslinked tissue calcified after the transplantation for 3 month. Inflammatory response in the surrounding tissue of the xenograft was evaluated by vascular-graft transplantation model. After the transplantation of the unmodified graft into femoral artery of minipig, M1 macrophage-related genes were increased as compared with the results in allograft-transplantation model. On the other hand, the gene expressions were significantly reduced in the case of the PEG-modified graft. From these results, the inflammatory response are effectively regulated by the PEG modification without losing the degradation activity. . [Acknowledgments] This research was supported by S-innovation project of JST.

Session No.: S34-04

Evaluation of MTA and Bioceramic as a Pulp Capping Agent in Swine

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Reparative dentin formation is essential for maintaining the integrity of dentin structure during disease or trauma. Most studies of pulp capping agents focused on the capacity of newly formed reparative dentin,while few studies explored the pulp through pathological analysis after pulp capping in vital pulp therapy. In this study, we investigated how the dental pulp respondto three popular clinically used pulp capping agents, i.e.,iRoot BP Plus, MTA and calcium hydroxide (CH), in miniature swine. We randomly assigned 26 premolars of 4 miniature swineinto 3 groups. Class I cavities with 2mm diameter of pulp exposures were prepared on the occlusal surfaces. Two months later, teeth were harvested, fixed and processed for histological analysis. Interestingly, both MTA and iRoot BP Plus group can form homogenous dentinal tubule like structure of reparative dentin with polarizing odontoblast layer on the pulp side. The thickness of the dentin bridge was about 0.50± 0.14mm and 0.19±0.04mm in MTA group and iRoot BP Plus group, respectively. Some specimens in MTA group showed that the newly formed calcium bridge was separated by the fibrous connective tissue. Additionally, the adjacent pulp tissue showed different levels of inflammatory response and over calcification in MTA group. However, there was only slight inflammatory response and no obvious calcification in theiRoot BP Plus group. In calcium hydroxide group, pulp necrosis occurred underneath the materials. The newly formed calcium bridge was loose and very random. Pulp chamber showed vascular congestion, infiltration of inflammatory cell and irregular calcification. This study demonstrated that calcium hydroxide is not good candidate for maintaining pulp tissue. Unlike MTA，iRoot BP Plus showed completely reparative dentin formation and served as pulp protection. Key words: vital pulp therapy, pulp capping agents, iRoot BP Plus, MTA, calcification.

Session No.: S34-05

Biodegradable Poly(Propylene Carbonate)-based Composite: An Alternative Biomaterial to Polylactic Acid

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Poly(lactic acid) (PLA) and other polyester-based polymers are broadly used in biomedical applications due to their favourable mechanical strength and biodegradable properties. However, the acidic properties of their degradation products may lead to clinical complications, such as inflamation, long-term osteoporosis and other unpredictable issues. In this study, we demonstrate the superior properties of the poly(propylene carbonate) (PPC)-starch composite as an alternative to polyester-based biomaterials. The degradation products of PPC-starch are mainly carbon dioxide and water; hence, the pH in the surrounding tissues of an implant fabricted from this composite does not decrease. Moreover, the mechanical strength of PPC-starch composites is tuneable within the range of 0.2 ± 0.03 MPa to 33.9 ± 1.51 MPa, by varying the starch content from 0 to 50 w%. PPC-starch composites are cytocompatible as osteoblast cells adhere and proliferate on their surface within 7 days. The long-term biocompatibility of PPC-starch was assessed via subcutaneous implantation in mice. The results of histological analysis demonstrated no symptom of inflammation for PPC-starch composite after eight weeks implantation, while the biodegradation of PLA led to massive immune cell infusion and inflammation. These results underline that PPC-starch is suitable for biomedical applications and can be used for the musculoskeletal tissue regeneration.

Session No.: S34-07

Ionic Conductive Hydrogel for Strain Generators and Sensors

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Hydrogels, because their properties such as high water content and physical characteristics that resemble the native extracellular matrix, are promising materials for cell culture and tissue engineering. Hydrogels swollen with ionic liquids are biocompatible ionic conductors, which can be used in devices to integrating biology and electronics. Here, we report an elastic, porous, ionic conductive hydrogel, similar with piezoelectric materials, which can conversion mechanical energy to electric energy. When the hydrogel immersed in the electrolyte, and compress the surface, positively charged hydrogel main network preferentially push the counter-ions flow and stop the co-ions flow, which leads to net movement of charge, creates synchronous ionic current. The density of streaming current was dependent by stress and concentration of electrolyte solutions. More than that, the direction and current density of the streaming current was changed because the polarizing and the ionisating of the main network chains when tuning the pH. This piezoelectric hydrogel offer new opportunities for designers of soft devices such as strain generators and sensors, and also use as artificial skin and muscle.

Session No.: S35-01 Keynote Speaker

Thermosensitive Pluronic-Poly (Alanine)-based Hydrogels for Tissue Engineering Matrix and Scaffolds

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Amine terminated PEO-PPO-PEO was used to synthesize a series of Pluronic-poly(alanine) (PLUF-PA) and Pluronic-poly(phenylalanine) (PLU-PPhe) copolymers. These polymers were prepared as thermosensitive hydrogels and exhibited decreased gelation concentration, extended in vitro and in vivo residence time, increased cell compatibility, and change in microarchitecture when compared to the native Pluronic. Taken together, the results suggest that this material may be suitable for various bio-applications, including tissue engineering scaffolds and drug carriers.

Session No.: S35-02 Invited Speaker

Designing Culture Microenvironments to Direct Stem-cell Fate in the Regulation of Cell Behaviors

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[1]Osaka Univeristy

In recent years, accumulating evidence suggests that modulation of intercellular communication and alterations in intracellular signals originating from cell behaviors are critical for regulation of cell-fate decision. In this study, we describe our novel strategies for creating a culture surface to control stem cell fate in the regulation of behaviors, as well as approaches to their underlying mechanisms of action. Dynamic changes of cell morphologies were investigated on dendrimer surfaces with different capacities for fibronectin adsorption by changing the generation numbers. The amount of adsorbed fibronectin on dendrimer surfaces increased with the generation number. In the case of hiPSCs examined in this study, hiPSCs on the 1st-generation (G1) dendrimer surface formed tightly packed colony with close cell-cell adhesions during division and migration; those on the 5th-generation (G5) dendrimer surfaces, formation of aggregated colony with ring-like structures occurred spontaneously. This subsequently resulted in cell migration and in activation of paxillin of hiPSCs. Cells on the G1 surface were maintained in an undifferentiated state, while those on the G5 surface exhibited the early commitment to differentiation toward endodermal fates. A similar dependence of cell–matrix and cell–substrate adhesions was also seen in cultures of hMSCs. It also found that the amount and structure of fibronectin affects lineage specification through the formation of cytoskeletons and focal adhesions in regulation of dynamic cell behaviors. These results indicate that control of the cell–surface interface in fibronectin adsorption and assembly represents a versatile approach to direct stem-cell fate. The current findings suggest that the dendrimer surface offers a model of designing a substrate, based on interactions between the microenvironment and cells, as a tool for directing stem-cell fate in an ex vivo stem cell culture system.

Session No.: S35-03 Invited Speaker

High Throughput Analysis of Individual T Helper Cells for Their Antigen-specific and Characteristic Responses

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Human CD4+ T helper cells play central roles in immune responses, adopting distinct functional patterns of cytokine release upon antigenic exposure. Exogenous factors such as cytokines modulate these patterns of functional responses, suggesting that populations of cells can exhibit specific responses. It remains unclear, however, if this population-level responses might arise in part from the dynamic modulation of responses by individual cells since single-cell technologies have previously resolved only integrated measures of function. Here, we use a single-cell co-culture assay to temporally resolve antigen-dependent, multiple cytokine responses exhibited by human CD4+ T cells ex vivo. Monocyte-derived human dendritic cells pulsed with peptides from common antigens (influenza, tetanus toxoid, cytomegalovirus, and Epstein-Barr virus) were co-incubated in arrays of microwells with individual autologous human CD4+ T cells, and the secretory responses were measured for three different analytes at two time points. The results show that the reported single cell assay can probe the "plastic" profile of functional responses from individual antigen-reactive T cells persistently stimulated by antigen-presenting cells.

Session No.: S35-04 Invited Speaker

Characterization and Serum-free Expansion of Endothelial Progenitor Cells on Surface Modified Polyhydroxyalkanoate Scaffold for Blood Vessel Tissue Engineering

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Repair and regeneration of vascular tissue is the important research topic of current biomedical engineering and regenerative Medicine. Many studies indicated that cells need to grow on the suitable extracellular matrix to show the particular functionality. In this study, we tested various surface modification methods to fix fibronectin or collagen on the biodegradable polymer surface (poly(3-hydroxybutyrate, PHB and poly(3-hydroxybutyrate-co-3-hydroxyvalerate), PHBV). Then, the cell lines (3T3 and L929) and primary cells (endothelial progenitor cells, EPCs and human umbilical vein endothelial cells, HUVECs) were cultured on the modified surface to explore the application potential of vascular tissue engineering. Our data showed that the surface of alignment PHB and PHBV films can be modified successfully by chemical methods based on Ninhydrin assay and contact angle assay. XPS assay also confirmed ECM has immobilized on the film. In addition, the WST1 assay, immunocytochemistry assay and SEM showed that the surface modified films performed excellent cell compatibility. The cells cultured on the surface modified films, the cell viable assay showed that the films had good biocompatibility. Taken together, our results demonstrated that PHB and PHBV films that were modified by the above chemical method and were fixed with suitable ECM can provide a potential artificial vessel for application of vascular tissue engineering.

Session No.: S35-05 Invited Speaker

Tissue Engineering Materials Based on Natural Polymer

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Tissue engineering is an interdisciplinary field combining biomaterials and medical sciences to improve the understanding of tissue pathology using principles that are applied to improve or sustain tissue function through the development of biological substitutes. Natural polymer are of substantial interests in various research fields due to their unique properties, such as safety, biocompatibility, hydrophilicity, biodegradability, etc. Various types of natural polymer-based tissue engineering materials were synthesized so far, such as polysaccharide-based (hyaluronic acid, alginate, chitosan, and cellulose), protein-based (collagen, gelatin), and deoxyribonucleic acid (DNA) –based. In our group, we used natural polymer such as bacterial cellulose, Silk sericin, chitosan etc. to fabricate a series of tissue engineering materials further to application in skin, neuron regeneration and bioartificial liver.

Session No.: S35-06 Invited Speaker

A Practical Using of Thai Silk Fibroin as a Biomedical Material

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Fibroin is a natural silk protein reported as a high potential biomedical material due to its compatibility and mechanical strength. Thai silk fibroin (SF) from cocoons produced by the Queen Sirikit Sericulture Center, Thailand, has been studied for its biocompatibilities to various kinds of cells and minimal tissue reaction and slow degradability in animal transplantation. Thai silk cocoons (Bombyx mori), Nangnoi Sisaket-1, extracted using either 9.3M LiBr solution or calcium chloride-ethanol solution (Ajisawa's reagent) showed similar physicochemical characteristics. The regenerated SF had average molecular weights in ranges of 242-256 kDa, with a polydispersive index of 2.5. Its major amino acids are Glycine (46.6-48.3%), Alanine (29.8-32.1%), Serine (5.1-8.1%), Tyrosine (4.5-5.0%), Valine, and others (2.3-2.6%), by weight, with an approximated pI of 4 (in water). Due to the nature of its mixed protein structures, the ATR-FTIR spectroscopy of the degummed silk fibers revealed 56.1% of beta sheet structure while the freeze-dried regenerated SF had 37.6-41.5% random coil 16.5-25.5% beta sheet, 22.7-30.9% beta turn and 8.5-15.7% helical structures. Percentages of beta turns and beta sheets increased during storage at 4℃ and upon treatment with ultra low temperature, high temperature, gamma irradiation, and chemicals such as ethanol or methanol. Thermal degradation of the regenerated Thai SF was at 283-290 ℃, lower than that of the degummed silk fibers (311-314 ℃). Due to complications and instability of the SF structure during sterilization, SF solution should be prepared at sterile condition, especially at the concentration higher than 2% (weight). Some biomedical applications of Thai SF will be discussed at the presentation. Biography A Ph.D. in Chemical Engineering (specialized in Biochemical Engineering) from Drexel University, PA, USA in 2002. She became one of the pioneer researchers in biomedical engineering and drug delivery system in Thailand. An author and co-author of more than 45 international papers, 8 Thailand

Session No.: S36-01 Keynote Speaker

Cell-derived ECM Engineering Toward Biomimetic Extracellular Microenvironment

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Extracellular matrix (ECM) is a complex but highly organized network of proteins and other macromolecules that play a critical role in cell adhesion, migration, and differentiation. To harness the benefits of ECM on regenerative medicine, morphogenetic signals that the biophysical ECM environments provide must be fully defined and decoded. While the effects of individual ECM macromolecules, i.e., collagen, fibronectin, and laminin on stem cells behaviors have been well documented, the real face of a physiologically more relevant ECM platform is much less understood, due partly to the lack of proper models. To address such issue, we have utilized cell-derived ECM (CDM) as an in vitro ECM model. Natural CDM is obtained from in vitro-cultured cells via decellularization process. In this work, CDM-based studies are presented: 1) angiogenesis, 2) cartilage and bone tissue regeneration, and 3) CDM modifications. Upon the critical role of ECM in the vascular system, studies of CDM-based 2D or 3D environment show excellent capillary formation in vitro and present significantly better wound healing in vivo. Secondly, polymer mesh scaffold was coated with CDM and subsequently processed for BMP-2 and TGF-b1 immobilization, respectively. Differentiation of human mesenchymal stem cells (hMSCs) into osteogenic and chondrogenic lineage was greatly improved via CDM-coated mesh group. Animal model studies also support a bioactive role of CDM in tissue regeneration. In addition, CDM is modified either for growth factor tethering or for matrix crosslinking. Heparin-conjugated matrix efficiently immobilizes VEGF and crosslinking agent changes physical property of CDM. VEGF-tethered matrix serves as a growth factor delivery carrier for angiogenesis. Matrices with different crosslinking densities are investigated via multi-lineage differentiation of hMSCs, along with in-depth characterizations of crosslinked matrix. Taken together, present works strongly indicate the validity of biomimetic CDM in tissue regeneration and enhance our understanding of cell-ECM interactions on specific ECM microenvironments.

Session No.: S36-02 Keynote Speaker

Cells and Extracellular Matrix Interactions in Tissue Regeneration

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ECM is a complex interlinked composite of collagenous molecules, non-collagenous molecules and water-rich mucopolysaccharide ground substance. Cells are integrated to their matrix via integrin and non-integrin receptors, which are utilized in the control of adhesion, migration, division, growth, anoikis, transdifferentiation and other cellular behaviour. A decellularized ECM maintains a niche, which can serve to maintain cell phenotype and encourage production of tissue specific matrix and functional properties. Preservation of the native ultrastructure and composition of ECM during the process of tissue decellularization is highly desirable. The procedure of decellularized ECM is to remove all cellular, nuclear components, any possible remains of chemical reagent, virus (inactivation) and to minimize any adverse effect on the composition, biological activity, and mechanical integrity of the remaining ECM. The structural and functional molecules of the ECM are in a state of dynamic equilibrium, and also provide the means by which cells communicate with each other and the external environment. Based on the principal and technology, we developed the decellularized porcine cornea approved by CFDA in 2015. After Lamellar keratoplasty, the corner ulcer was repaired and the graft was integrated with the host cornea and the gradually recovery of corneal transparency. We developed another kind of ECM which is self-produced ECM. During the long term culture, dissociated cells intermixed in vitro tend to group themselves after cell-cell interaction and then form a cell aggregate (CA).The advantage of CA is composed of self-assembling cells, self-produced ECM, and maintained the cell-cell relationship after culture. We successfully made the CA system to repair periodontal, cartilage.

Session No.: S36-03 Invited Speaker

Nanoengineered Extracellular Matrix for Scaled-up Culture of Human ES/iPS Cells

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Human pluripotent stem cells (hPSCs), i.e., embryonic and induced pluripotent stem cells (hESCs and hiPSCs, respectively) hold great potential for industrial and clinical applications. To cure patients who have serious diseases in large tissues (e.g., heart and liver), large quantities (more than 108 cells) of quality-controlled hPSCs must be prepared for further differentiation procedures for targeted tissue cells and better transplanted cell engraftments at the curing area in a patient. However, the use of traditional two-dimensional (2D) culture systems (i.e., culture dishes, multi-well plates or flasks) to generate high quality and large quantity hPSCs is not realistic due to the requested huge space and medium as well as too labor-intensive procedures. For this purpose, it is necessary to establish 3D culture systems to allow for an increase in the number of cells per unit volume. Although the numbers of 3D hPSC culture systems have been reported, they also showed a number of drawbacks, such as insufficient cell growth, unexpected cell differentiation, scalability, and hydrodymanic shear stress. To address these issues, we develop a new type of extracellular matrix (ECM) enabling efficient promotion of hPSC self-renewal and prevention of undesired differentiation during culture. Nanofibers have unique advantages over conventional ECMs for increasing interaction with cells, and can be fabricated with defined materials. Thus, nanofiber matrices would be suitable for good manufacture’s practice. However, since nanofibers are mechanically fragile, it is difficult to apply for establishing 3D cell culture system with them alone. Therefore, we introduce a microfiber matrix as a backbone to improve mechanical stability; we named this “Fiber-on-Fiber” matrix. This matrix allows hPSC expansion at a much higher density than conventional culture dishes with the same amount of culture medium, while maintaining pluripotency, robust proliferation, and normal karyotype.

Session No.: S36-04

Simulation of Native Tissue Mechanics by Welding of Electrospun Fibers

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Tissue engineering is an emerging technology for replacement grafts. In order to recapitulate mechanical functionalities of the native tissue, we hypothesize that simulation of the native matrix organization will recreate the structure-function relationship. As many tissues, such as ligament and tendon, are composed of crosslinked collagen fibers in parallel crimp structures, the goal of this study was to develop scaffolds with controllable fiber alignment, crimp, and crosslinking. Electrospinning of polylactid acid (PLA) was employed to generate parallel fibers and crimp was induced by briefly heating the material over its glass transition temperature, as we previously demonstrated. This crimp morphology contributes to nonlinear mechanics of the bulk material, similar to native properties. Fiber crosslinking was achieved by embedding the material in polyvinyl alcohol (PVA), heat welding, and removal of the embedding medium, thus preventing alterations of fiber morphology. Scanning electron microscopy revealed that fibers maintained their parallel and crimp morphology after PVA removal. Similar cell morphologies were observed in the heat welded group when compared with the non-treated controls, also representing preservation of fiber structure. Mechanical testing parallel and perpendicular to the fiber direction found increases of bulk material stiffness when fiber welding was done around the melting temperature of the material, indicative of fiber crosslinking. Current studies are ongoing to develop a mechanical model describing the mechanical contribution of each of these parameters (alignment, crimp, fiber-fiber interactions) to facilitate future scaffold designs. The ability to recapitulate tissue functionality in a biocompatible material allows a fully functional graft that enhances cell growth while maintaining tissue integrity.

Session No.: S36-05

Engineered Phage Based Matrix Stiffness Modulating Osteogenic Differentiation

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Although it is known that specific biochemical cues in tissue extracellular matrices (ECM) play a critical role in regulating cellular growth processes and their fate, the role of physical cues of them such as stiffness in guiding the fate of resident stem cells has not been well studied so far. In this study, we have demonstrated engineered phage mediated matrix controlling stiffness for various applications over conventional tissue engineering materials by exploiting its physical and biological structural features (such as the phage's self-assembling, selfreplicating and evolving nature). We modified M13 phages to express biotin-like peptides (HPQ) and/or integrin binding peptides (RGD) on their major and minor coat proteins. The stiffness of matrix was controlled by cross-linking the engineered phage with different concentrations and compositions of streptavidin and polymer mixture. Then, we verified that osteogenic differentiation could be controlled according to the rates of stiffness of the constructed phage matrix. Osteogenic gene expressions through mRNA expression quantification and protein activity assays showed that they were specifically increased when bone stem cells were cultured on the M13 matrix with adequate stiffness. Our phage matrices, which can be easily functionalized with various ligands and growth factors to enhance the stiffness modulation using other chemicals, may be used as a convenient tissue matrice platforms for controlling stem cell expansion and differentiation. [This work was supported by the National Research Foundation of Korea Grant funded by the Korean Government(NRF-2014S1A2A2027641)]

Session No.: S36-06

Functionalized Surfaces by Vitronectin-binding Heparan Sulfate

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Functionalizing medical devices with polypeptides to enhance their performance is important for improved clinical success. The extracellular matrix (ECM) adhesion protein vitronectin (VN) is particularly effective as a coating material however the chemistry used to attach VN often reduces its bioactivity. In vivo, VN is bound into the ECM in a sequence-dependent manner with heparan sulfate (HS) glycosaminoglycans. Therefore, we reasoned that sequence-based affinity chromatography could be used to isolate a targeted, VN-binding heparan sulfate (HS) fraction (HS9+ve) that could be used as a coating material to capture VN to implant surfaces. Binding avidity and specificity of HS9+ve was confirmed by ELISA and SPR-based assays. Plasma polymerization of allylamine (AA) to tissue culture-treated polystyrene (TCPS) surfaces was used to more efficiently capture and present HS9+ve as determined by radiolabeling and ELISA assays. TCPS was used as a proof-of-concept material to assess the utility of this coating strategy. HS9+ve-coated TCPS avidly bound VN and this VN-functionalized surface supported the robust attachment, expansion, and maintenance of human pluripotent stem cells. Compositional analysis demonstrated that 6-O- and N-sulfation, and lengths greater than 3 disaccharide units (dp6) are critical for VN binding to sugar-coated surfaces. Importantly, HS9+ve surface coating significantly reduced the threshold concentration of VN required to create an optimally bioactive surface. This property was validated by the stringent test of long-term maintenance of human pluripotent stem cells on the functionalized surface. Hence, affinity-purified heparan sugars can effectively coat materials to selectively and efficiently bind bioactive factors for medical applications

Session No.: S37-01 Keynote Speaker

New Gene Editing Technique to Save Sight

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The field of regenerative medicine holds considerable promise in treating numerous ocular degenerative diseases that are currently intractable. For successful ocular tissue regeneration, it is indispensable to either provide the health cells exogenously or restore function of sick cells, which enable to maintain the functional vision efficiently. New gene editing technology, known as Clustered Regularly-Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein (Cas), is considering as power tools to achieve these objects by offering the different types of ocular cells with fully cellular function for preventing the blindness and even restoring the vision in patients. Nonetheless, despite these promising applications it is clear that such approaches for CRISPR/Cas delivery in vivo are not currently applicable as a human therapy. Our group has successfully explored viral-mediated delivery of essential CRISPR/Cas components to retinal cells in vivo. Using a transgenic fluorescent mouse model, we have definitive evidence for CRISPR/Cas-mediated gene editing of retinal cells. Moreover, CRISPR/Cas genotoxicity is a significant consideration in clinical applications and, regardless of fidelity, it is foreseen that the overall chances of eventually cutting off-target sites which result in cytotoxicity, would accumulate with time. To address this issue, we have rationally designed self-destructing “kamikaze-CRISPR” system that enables destruction of CRISPR enzymes after initial activity. This work will lay the foundation for a new generation of gene therapy: “direct gene editing” for the inherited degenerative ocular diseases.

Session No.: S37-02 Keynote Speaker

Injectable in situ-Forming Hydrogels as Drug and Cell Carrier

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Research on injectable in situ-forming hydrogels has been conducted in diverse biomedical applications for a long period. These hydrogels exhibit sol-to-gel phase transition in accordance with the external stimuli such as temperature change. Also they have the distinct properties of easy management and minimal invasiveness via simple aqueous state injections at target sites which replace the traditional surgical procedures. Currently, numerous polymer materials have been reported as potential stimulus-induced in situ–forming hydrogels. Here, a comprehensive overview of these rapidly developing materials has been outlined. In situ–forming hydrogels formed by electrostatic and hydrophobic interactions as well as their mechanistic characteristics and biomedical applications as drug and cell carrier have also been discussed.

Session No.: S37-03 Invited Speaker

Genetic Correction for Mitochondrial Mutation in Induced Pluripotent Stem Cell Model

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Optic neuropathies are blinding diseases characterised by damaged retinal ganglion cells (RGCs), specialised neurons which relay visual information obtained by the retina for processing in the brain. In particular, Leber's Hereditary Optic Neuropathies (LHON) is the most common mitochondrial-DNA (mtDNA) disease and is caused by homoplasmic mtDNA mutations. It is a blinding disease that is characterised by loss of RGCs. Our aim is to use human induced pluripotent stem cell (hiPSC) technology to model LHON. In recent years, the field of hiPSC disease modeling has focused on the use of isogenic control, i.e. by correcting the genetic defects to verify the disease phenotypes observed in the hiPSC model. However, the difficulty of genetically manipulating mtDNA is an obstacle for using hiPSC isogenic controls to model homoplasmic mtDNA diseases. Using an episomal reprogramming method, we generated patient-specific iPSCs from control and LHON patients. Here we report the use of cybrid technology to correct mtDNA mutations and generate hiPSC isogenic controls for modeling LHON. We performed genotypic analysis to confirm the correction of mtDNA mutation in cybrid hiPSCs and microsatellite analysis to confirm the cell origin. Characterisation of these iPSCs demonstrates that the cells are positive for pluripotent markers TRA-160 and OCT4 and retain abilities to differentiate in vitro into cells representative of the three germ layers. Following RGC differentiation, we showed that LHON-specific RGCs exhibit disease phenotypes that recapitulate those observed in LHON patients. Notably, these phenotypes are alleviated in the cybrid hiPSCs, suggesting that the correction of mtDNA mutations by cybrid technology can reverse the diseased phenotypes observed in this hiPSC model. In summary, our study provided a novel method to generate mtDNA mutation-free hiPSC isogenic control, which can be applied to hiPSC modeling of mitochondrial diseases.

Session No.: S37-04 Invited Speaker

Toward "Chromosome Therapy": Cell-autonomous Correction of Ring Chromosomes in Human iPSCs

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One promising application of iPSC technology is the potential use of patient-derived cells to correct defects by transplantation via regenerative medicine technology. If a patient has a monogenic disorder, gene therapy or new gene-editing techniques can be used to correct the genetic defect in these cells prior to transplantation. However, the use of stem cell therapy after gene correction is currently limited to monogenic diseases with an altered or mutated gene. Current gene-editing techniques are not feasible for large chromosomal abnormalities. Here we generated human iPSCs from patient fibroblasts containing ring chromosomes, which are structural aberrations commonly associated with birth defects, mental disabilities and growth retardation and formed after fusion of the long and short arms of a chromosome, and are sometimes associated with large terminal deletions. Reprogrammed cells lost the abnormal chromosome and duplicated the wild-type homologue through the compensatory uniparental disomy (UPD) mechanism. The karyotypically normal iPSCs with isodisomy for the corrected chromosome outgrew co-existing aneuploid populations, enabling rapid and efficient isolation of patient-derived iPSCs devoid of the original chromosomal aberration. Our results suggest a fundamentally different function for cellular reprogramming as a means of ‘chromosome therapy’ to reverse combined loss-of-function across many genes in cells with large-scale aberrations involving ring structures. In addition, our work provides an experimentally tractable human cellular system for studying mechanisms of chromosomal number control,which is of critical relevance to human development and disease.

Session No.: S37-05 Invited Speaker

Correcting Disease Causing Mutations in iPSC Models of Retinal Dystrophies

Sandy Hung[1], Maciej Daniszewski[1], Stacey Jackson[1], Helena Liang[1], Lisa Kerns[1], Ruchira Singh[2], David Mackey[3], Raymond Wong[1], Alex Hewitt[1], Alice Pébay[1]

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Breakthroughs in cellular and molecular technologies have led to the ability to generate induced pluripotent stem cells (iPSCs) from adult somatic cells, which can be subsequently differentiated into cells of interest. This offers the unique ability to interrogate pathological processes in specific cell types such as retinal cells, which cannot be easily obtained pre-mortem. Furthermore, the adaptation of Clustered Regularly Interspersed Short Tandem Repeat (CRISPR) and CRISPR-associated (Cas) protein technology to mammalian cells has enabled the direct editing of genetic variants with high fidelity. In our study, we used CRISPR/Cas9-mediated gene editing technology to correct and assess two specific mutations, which cause two distinct blinding retinal diseases- Best Disease and Doyne Honeycomb Retinal Dystrophy. Genetic correction of retinal dystrophy iPSC models will help us assess the value of genetic correction in rescuing disease phenotype and will be an important a step towards the development of next generation of gene therapies for retinal diseases.

Session No.: S38-01 Keynote Speaker

Secretome of Human Fetal Mesenchymal Stem Cells (MSCs) Ameliorates Replicative Senescence of Human Adult MSCs

Gang Li[1], Bin Wang[1], Wayne Yuk-Wai Lee[1], Jinfang Zhang[1], Xiaohua Jiang[1]

[1]The Chinese University of Hong Kong

Autologous mesenchymal stem cells (MSC) is widely used cell source for cell-based tissue repair and regeneration, but replicative senescence and the associated loss of cellular activity during in vitro expansion limit their therapeutic potential. How to preserve or even enhance the proliferation and differentiation ability of MSC from aged donors without genetic modification remains a challenge to meet clinical need. MSC isolated from human fetal tissues (hFMSC) exhibit higher proliferation and differentiation activities even in prolonged in vitro culture, which might modulated by autocrine/paracrine action. In the present study, we hypothesized that the bioactive factors secreted by hFMSC, collectively named as hFMSC secretome (HFS), could possess beneficial effect on human adult MSC (hAMSC) undergoing replicative senescence, thus promoting their capability of proliferation and differentiation. HFS was prepared by centrifugation of hFMSC conditioned medium, followed by column-based concentration, and the total protein content of the HFS was quantified to standardize treatment concentration. When compared with hAMSC secretome (HAS), HFS treatment significantly reduced senescence associated-β-galactosidase (SA-β-gal) expression and activity (senescence marker), and enhanced cell proliferation and osteogenic differentiation potential of hAMSC in prolonged in vitro culture. Cellular studies revealed concomitant activation of sirt1 and foxo3a in hAMSC after HFS treatment, which was associated with upregulation of p21 and downregulation of bax and p53. The changes of these senescence associated markers suggested that HFS, but not HAS, could ameliorate replicative senescence of hAMSC in vitro. In nude mice, HFS pre-treatment restored the osteogenic ability of senescent hAMSC. Tumor xenograft model revealed that HFS did not promote tumor growth. In conclusion, this study suggests that HFS could be an effective and safe method to overcome replicative senescence and facilitate the therapeutic potential of hAMSC.

Session No.: S38-02 Invited Speaker

Guiding, Maintaining and Resonating Epigenomic Modulations in Germ Cells, Stem Cells and Beyond

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Illuminating the machineries and factors that guide epigenetic modifications explains how cells respond to environmental cues to make cell fate decisions. DNA methyltransferase 3-like (DNMT3L), which is a regulatory co-factor that does not exhibit enzymatic activity but significantly influences epigenomic signatures, serves as one of the top candidate molecules for deciphering epigenomic guiding mechanisms. DNMT3L is predominantly expressed in developing germ cells and embryonic stem cells. On top of facilitating de novo DNA methylation and interpreting chromatin context, our lab discovered that DNMT3L also facilitates modulations at chromatin levels to silence newly infected retroviruses and endogenous retroviral sequences. During mouse male germ cell development, DNMT3L is expressed in two stretches of quiescent stages, including embryonic prospermatogonia stage and a portion of quiescent postnatal spermatogonial stem cells (SSCs) that are important for maintaining long-term fertility. DNMT3L is required for guiding genome wide epigenetic signature establishment in embryonic prospermatogonia, and for delicately balancing the cell cycling and quiescence of SSCs by preventing over-proliferation and premature stem cell exhaustion. The lasting effect of DNMT3L-mediated epigenetic modulations can be observed long after DNMT3L is silenced, for example, in mouse embryonic fibroblasts (MEFs). Dnmt3l KO MEFs have higher levels of permissive H3K27ac, lower levels of repressive H3K9me3 and H3K27me3 modifications, and increased expression of development-associated genes compared to wild-type MEFs. When taking Dnmt3l KO MEFs as donor cells for somatic cell nuclear transfer (SCNT) mediated reprogramming, we observed a significant 43% increase of cloned blastocysts formation rate, and much better cloned embryo quality compared to using wild-type MEFs as SCNT donor cells, therefore facilitates embryonic stem cell derivation from SCNT cloned embryos. Our ongoing studies will decipher the multiplayers of epigenetic regulations in tissue homeostasis, with anticipated outcomes that will help disease diagnosis, and provide insights and stem cell sources for developing therapeutic strategies.

Session No.: S38-03 Invited Speaker

The Extracellular Matrix in Stem Cell Differentiation and Reprogramming

Yihua Loo[1], Nina Ma[1], Lim Jia Kai[1], Lu Hong Fang[1], Karthikeyan Narayanan[1], Du Chan[1], Leong Meng Fatt[1], Lim Tze Chiun[1], Chua Ying Ping[1], Andrew Wan[1]

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As a major component of the cellular microenvironment, the extracellular matrix (ECM) exerts a profound influence on cellular processes such as adhesion, proliferation, differentiation and reprogramming. Looking beyond biochemical and topographical cues, recent studies demonstrate the dynamic collaboration between ECM and cellular context that has profound implications on stem cell gene expression and behaviour.

For the past ten years, our lab has developed a variety of novel scaffolds to evaluate how the manner and context in which ECM is presented can influence cellular reprograming and stem cell differentiation. Interfacial polyelectrolyte fibers encapsulating ECM molecules can be patterned into aligned scaffolds that promote cell alignment. This in turn enhances stem cell differentiation. The 3D patterning of fibers also enables us to recreate niche microenvironments for tissue engineering. We have also pursued stem cell differentiation using decellularized ECM and xeno-free cellular reprogramming strategies. More recently, we utilized electrospun polystyrene (ESPS) scaffolds to engineer a 3D glioblastoma model. The 3D context allowed us to demonstrate that ECM promotes cancer stemness in collaboration with tumor cell growth, as well as to probe the relevant cellular interactions that mediate this collaboration. By shedding light on how the ECM affects stem cell proliferation and differentiation, we hope to build better tissue models for regenerative medicine and drug screening.

Session No.: S38-04 Invited Speaker

Effect of Micropatterned Bioceramics on the Growth and Differentiation of Adipose Derived Stem Cells

Yogambha Ramaswamy[1], Seyediman Roohaniesfahani[2], Genevieve Madafiglio[2], Frank Chang[2], Furong Zhao[2], Hala Zreiqat[2]

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Ordered nano/micro patterned surfaces have attracted considerable attention as means to enhance the bioactivity of biomaterials. Lithography, chemical etching, mechanical punching and self-assembly are some of the techniques employed to produce patterned polymeric or metal surfaces. However, the challenge remains in producing patterned surfaces with brittle materials such as ceramics. The aim of this study is to develop a simple cost effective sustainable technique to produce ordered micropatterns on ceramic substrates. Hydroxyapatite (HAp) nanoparticles were synthesized by wet chemical precipitation method and HAp bioceramic substrates with ordered micropatterns were fabricated. Three unique and distinct micropatterned structures were developed and the unmodified flat surface was used as the control. The effect of the different micropatterns on the bioactivity of the ceramics was evaluated using primary human adipose derived stem cells (ASCs). We demonstrated that variation in the microtopographic pattern induced marked differences in the cell morphology and osteogenic differentiation of ASCs. The cellular responses to the unique patterns developed in this study shows that inducing the topographical changes to the currently available ceramic implant materials may be important design criteria for enhancing the bioactivity of currently available ceramic implants.

Session No.: S38-06

The Crucial Role of MMP-13 in Remodeling of Type I Collagen During Osteogenic Differentiation of Human Adipose-derived Stem Cells

Yoshie Arai[1], Bogyu Choi[1], Byoung Ju Kim[1], Sunghyun Park[1], Inbo Han[1], Soo-Hong Lee[1], Eun-Mi Park[1], Byung-Hyun Cha[1]

[1]Cha University

Matrix metalloproteases (MMPs) are essential for the intra- and extra-cellular biology of stem cells, such as proliferation, survival, morphogenesis, and differentiation through extracellular matrix remodeling. Type I collagen (Col I) is a major component of bone tissues, which is known to promote osteogenic differentiation of human adipose-derived stem cells (hASCs). However, the mechanism of the effects of Col I on osteogenesis is still not clear. It has been proven that cell-matrix interaction plays an important role in osteogenic differentiation of hASCs. In this study, we evaluated that the expression of MMPs and integrins during osteogenic differentiation in presence of Col I. As a result, Col I triggered the high level of expression of MMP-13 and ITGA3. The mRNA level of ITGA3 was reduced by MMP-13 silencing using siRNA, while the mRNA level of MMP-13 was not affected by a knockdown of ITGA3. Col I also induced RUNX2 translocation into nucleus. It is known that RUNX2 is transcription factor which bind to MMP-13 promoter. We suggest that MMP-13 initiated and enhanced osteogenic differentiation of hASCs through the activation of focal adhesion molecules including ITGA3 in the presence of Col I. Furthermore, nuclear translocated RUNX2 by hASCs-Col I interaction will regulate MMP-13 expression. Consistent with in vitro experiment, osteogenic capacity of MMP-13 overexpressed hASCs was increased in the presence of a Col I after 14 days of mouse subcutaneous transplantation. These results collectively suggest that hASCs-Col I interaction can enhance osteogenic differentiation both in vitro and in vivo through Col I/MMP-13 positive feedback loop.

Session No.: S39-01 Keynote Speaker

Designed Biomaterials for Regeneration of Cartilage/Subchondral Bone and Mediation of Cell Migration

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The processes of tissue regeneration and remodeling depend strongly on the cell migration and differentiation. However, the processes have not been clearly clarified so far. Indeed, the cell migration is a very significant process in many physiological and pathological events such as embryonic development, morphogenesis, angiogenesis, wound healing, immune response, and tumor metastasis. Therefore, it would be very meaningful to study the cell migration behaviors in vitro by designing specific materials, especially the materials with gradient chemical, physical and/or biological cues, to understand the factors influencing cell mobility and further develop new strategy for designing regenerative materials. We have designed a hydrogel-filled scaffold loaded with genes encoding cell growth factors and stem cells, yielding bioactive composites which can simultaneous regenerate cartilage and subchondral bone in vivo. More recently, we have been focusing on the following topics in terms of gradient biomaterials and cell migration: (1) preparation and characterization of several types of gradient biomaterials with continuous changes in grafting density, chain length and swelling properties; (2) Influence of physiochemical properties of gradient biomaterials on cell migration in terms of rate and direction; (3) directional migration of cells under the guidance of gradient cues; (4) selective adhesion of cells and directed migration; and (5) differentiation of mesenchymal stem cells in a spatially controlled manner. Acknowledgements This study is financially supported by the Natural Science Foundation of China (21434006, 21374097). References 1. W. Wang, B. Li, J.Z. Yang, L. Xin, Y.L. Li, H.P. Yin, Y.Y. Qi, Y.Z. Jiang, H.W. Ouyang, C.Y. Gao. Biomaterials, 2010, 31: 8964-8973. 2. J.D. Wu, Z.W. Mao, C.Y. Gao. Biomaterials, 2012, 33, 810-820. 3. L.L. Han, Z.W. Mao, J.D. Wu, Y. Guo, T.C. Ren, C.Y. Gao. Biomaterials, 2013, 34: 975-984. 4. T.C. Ren, S. Yu, Z.W. Mao, C.Y. Gao. Biomaterials 2015, 56: 58-67

Session No.: S39-07 Keynote Speaker

Cadherin-Matrix Engineering in Cell-recognizable Biomaterials

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Recently, embryonic stem (ES) and induced pluripotent stem (iPS) cells have shown remarkable potential to treat human diseases. Biomaterials are rapidly being developed as powerful artificial microenvironments to study and control stem-cell fate, such as proliferation and differentiation. Our recent advances include biomaterial-based artificial extracellular matrix formed by immobilizing cell-recognizable molecule, growth factors and cytokines. Here, we propose “Cadherin-Matrix Engineering” for new frontier of cell-recognizable biomaterials for construction of “Cell-cooking plate” for ES/iPS cells technology using chimeric proteins of cell adhesion molecules (e.g., E-cadherin, N-cadherin, or VE-cadherin).

Our novel E-cadherin-based engineered extracellular matrix showed fascinating results: (1) highly homogeneous differentiation of definitive endoderm cells under single-cell level; (2) uniform distribution of growth factors resulted in controlled differentiation even in lower concentration of free culture due to the absence of serum and feeder layers; (4) highly functional hepatocytes within soluble factors; (3) completely defined and xeno-short period of differentiation; (5) striking effect of matrix-dependent cell sorting for isolation and enrichment of mature hepatocytes for possible elimination of contaminated and poorly differentiated cells; (6) the unique opportunity for continuous monitoring of cellular behavior in different stages of differentiation.

Taken together, our novel recombinant ECM is advantageous for generating homogeneous population of differentiated cells without any enzymatic stress and cell sorting, suggesting that the improved method of differentiation is highly promising for clinically significant adult cells (hepatocytes, cardiomyocytes, or pancreatic cells). We established a novel biomedical field in cadherin biology named as “Cadherin-Matrix Engineering” which can be applied to “Cell-cooking Plate” for ES/iPS cells in regenerative medicine.

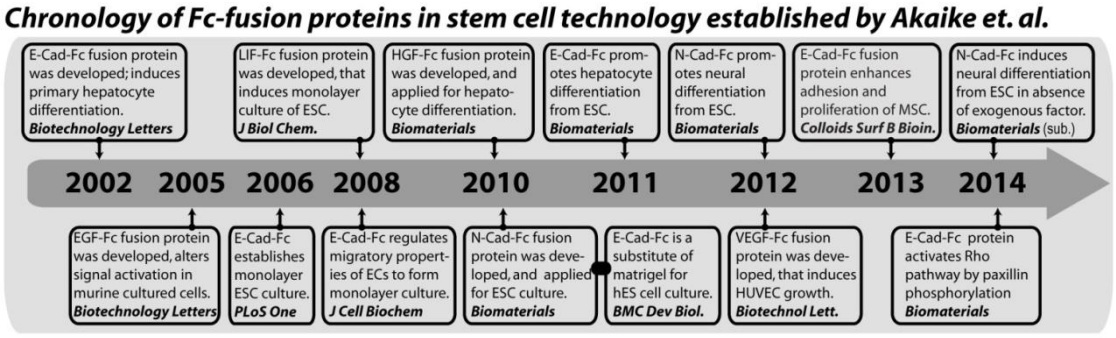

Table: Chronology of development of Fc-chimeric protein in Cadherin-Matrix Engineering for “cell-recognizable biomaterials”.

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Session No.: S39-02 Invited Speaker

Evaluation of Adhesion, Proliferation and Differentiation of Human Adipose-derived Stem Cells on Keratin Biomaterials

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Controlled adhesion and continuous growth of human adipose stem cells (hASCs) are essential to the delivery of hASCs on films in tissue engineering applications. The main goal of this study is to develop keratin substrates to actively control adhesion and proliferation of hASCs. Keratin, isolated from human hair, gained researchers' interests due to its intrinsic ability to interact with different cells. Keratin has the potential to serve as a controllable extracellular matrix protein and can be used to demonstrate cell mechanism and cell matrix interaction. However, the effects of keratin on stem cells have not been reported. In the present study, the effects of keratin on human adipose-derived stem cells (hASCs) were demonstrated. Relative to untreated culture plates, results showed that keratin coating substrates promoted cell adhesion and proliferation to above cell lines. Keratin also improved hASCs adhesion, proliferation, and enhanced cell viability. Evaluation of gene markers and protein levels showed that adipogenic, osteogenic and chondrogenic differentiations of hASCs can be successfully induced, which revealed that keratin did not influence the stemness of hASCs. In addition, keratin improved adipogenic differentiations in terms of up-regulations in lipoprotein lipase, peroxisome proliferator-activated receptor gamma and CCAAT-enhancer binding protein alpha. The osteogenic markers: type I collagen, runt-related transcription factor 2, and vitamin D receptor were also up regulated on keratin substrates. The chondrogenic marker SOX9 was also up-regulated on keratin coating. Therefore, keratin can serve as a biologically derived material for surface modification for biomedical purposes. The combination of keratin with stem cells may be a potential candidate for tissue repair in the field of regenerative medicine.

Session No.: S39-03 Invited Speaker

Cell-derived Decellularized Matrix Mimicking Native Ecm Dynamics During Tumor Progression for Tumor Tissue Engineering

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Malignant tumors are one of the leading causes of death in developed nations. Malignant tumor has been focused as cellular diseases caused by genetic mutations. Recently, it has been revealed that extracellular microenvironment, especially extracellular matrix (ECM), can influence cellular functions such as chemoresistance in tumor. “Tumor tissue engineering” is required for the applications of anti-cancer drug screening and basic tumor biology. In tumor tissues, ECM is dynamically remodeled to regulate cellular functions according to their malignant levels. Therefore, cell culture substrates mimicking ECM at different malignant levels should be provided for tumor tissue engineering. Here, I prepared “staged tumorigenesis-mimicking matrices” which were the substrates mimicking native ECM in tumor tissue at each malignant level and examined the chemoresistance of colorectal tumor cells on these matrices. “Staged tumorigenesis-mimicking matrices” were prepared by the decellularization following the culture of colorectal tumor cell lines, HT-29 (invasive), SW480 (non-invasive), and CCD-841-CoN (benign). Fresh HT-29 and SW480 were cultured and were exposed to 5-fluorouracil (5-FU) on staged tumorigenesis-mimicking matrices. Both HT-29 and SW480 exhibited the highest 5-FU resistance on invasive cell-derived matrices without the promotion of cell growth. Survival signal molecule, Akt, were exhibited the highest phosphorylation level in fresh HT-29 but not SW480 on invasive cell-derived matrices, suggesting that Akt activation was contributed to 5-FU resistance on invasive cell-derived matrices in part. Additionally, the expression levels of drug-efflux transporters, ABCB1 and ABCC1, increased on invasive cell-derived matrices, suggesting that rapid exclusion of 5-FU from the cells also contributed to 5-FU resistance on invasive cell-derived matrices. Finally, I found that JUN expression increased on invasive cell-derived matrices to promote ABCB1 expression. Conclusively, “staged tumorigenesis-mimicking matrices” will become preferred cell culture substrates for in vitro analysis of comprehensive ECM roles in chemoresistance and the screening and pharmacokinetic analysis of anti-cancer drugs.

Session No.: S39-04 Invited Speaker

Functionalized Graphene Oxide Nanosheets for Stem Cell Applications

Guan-Yu Chen[1], Jia-Wei Yang[1], Sheng-Jen Cheng[1], Yu-Chih Shen[1], You-Rong Lin[1]

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Graphene oxide (GO) has attracted significant interest as a template material for multiple applications due to its two-dimensional nature and established functionalization chemistries. Currently, as-synthesized GO nanosheets are employed directly to this end with no structural modifications. Here, we induce a phase transformation (oxygen clustering) in GO and demonstrate its benefits for one such application – stem cell differentiation. We utilize the modified form of GO to generate a uniform surface that allows for quick and efficient diffenentiate of stem cell to specific lineage. We also show that the enhanced efficiency stems from improved functionalization of GO with peptides as a result of chemical changes induced by oxygen clustering in GO. Overall, our work highlights a general route to improve functionalization of GO for various applications.

Session No.: S39-05

Enhanced Bonding Strength of a Biocompatible Tissue Adhesive Based on Hydrophobically-modified Gelatin

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For the treatment of pulmonary air leaks and anastomotic sites between living tissues, surgical sealants have been widely used in clinical field. Fibrin sealant is a typical sealant which consists of human blood components, however, it does not possess sufficient sealing effect because of its low interfacial bonding strength to tissues. Therefore, the molecular design of a tissue sealant which can adhere to a living tissue and organs under wet environment during surgery is required. We previously reported that tissue adhesives containing hydrophobically modified porcine-derived gelatins showed excellent bonding strength onto fresh vascular media compared with non-modified gelatin. However, porcine-derived gelatin solution with high concentration has low fluidity at room temperature because of its high contents of imino acids such as proline and hydroxylproline and is required to heat to use as a sealant. In this study, we have chosen Alaska pollock-derived gelatin (ApGltn) instead of porcine-derived gelatin as a base material because it has low transition temperature due to its low contents of imino acids. We synthesized cholestery group-modified ApGltns (Chol-ApGltn) with various modification ratios and evaluated their sealing effects on fresh vascular tissue (blood vessel) by combining Chol-ApGltns with polyethylene glycol-based 4-armed crosslinker (4S-PEG). Burst strength increased with increasing degree of Chol modification up to a maximum value of 8.3 mol% (8.3Chol-ApGltn). The highest burst strength of the 8.3Chol-ApGltn/4S-PEG adhesive was 341.3 ± 77.5 mmHg, which was 4- and 12-fold higher than that of the original ApGltn/4S-PEG and commercial fibrin adhesives, respectively. The 8.3Chol-ApGltn/4S-PEG adhesive swelled only slightly in saline (1.1-fold as compared to commercially prepared adhesive). Furthermore, tissue migration into the 8.3Chol-ApGltn/4S-PEG adhesive and subsequent biodegradation was observed following implantation in mice subcutaneous tissue for 4-8 weeks. These results suggest that the 8.3Chol-ApGltn/4S-PEG adhesive has potential for biomedical applications in the field of cardiovascular and thoracic surgery.

Session No.: S39-06

Laminin Facilitates Skeletal Muscle Myoblasts Growth Properties on 2D and 3D Culture Conditions

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The extracellular environment of human skeletal muscle maintains a strict population balance between contractile myoblasts and non-contractile fibroblasts. However, overgrowth of fibroblasts during in vitro culture makes it challenging to produce myoblast-enriched populations for clinical applications. Laminin, a major extracellular matrix protein in muscle, shown to enhance the proliferation, migration, and differentiation of myoblasts. This study was aimed to evaluate the effect of laminin on growth properties i.e. attachment, proliferation and migration of co-cultured myoblasts and fibroblasts on 2D and 3D culture conditions. Polystyrene surface and electrospun polymethylmethacrylate (PMMA) nanofiber scaffold was used for 2D and 3D culture, respectively. The human skeletal muscle was collected from three consented patients undergoing amputation and isolated a mixed population of myoblasts and fibroblasts. It was found that myoblasts preferentially attach on laminin-coated 2D culture surface, and demonstrate a dynamic movement compared to fibroblasts. Moreover, myoblast proliferation significantly increases on laminin-coated surface compare to that on uncoated surface. In contrast, fibroblast proliferation remains unchanged on both surfaces. Similarly, laminin-coated PMMA scaffold enhance myoblast attachment, proliferation and migration compare to non-coated PMMA scaffolds. Notably, myoblast-enriched population during expansion was achieved on laminin-coated 2D and 3D culture conditions due to the enhancement of myoblast proliferation. Therefore, laminin provides an opportunity to produce myoblast-enriched population during expansion and develop myoblast-enriched skeletal muscle tissue substitute for therapeutic applications.

Session No.: S40-01 Invited Speaker

Engineering Human Skeletal Muscle Biosynthetic Tissues Using Transdifferentiated Myocytes

Nicolas Christoforou[1], Selwa Boularaoui[1], Khaled Abdel--Raouf[1], Saradalekshmi Radha[1], Noaf Alwahab[1], Cesare Stefanini[1], Jeremy Teo[1]

[1]Khalifa University

Our main objective was the assembly of 3D engineered human skeletal muscle tissue that accurately resembles native in vivo muscle tissue for studying muscle physiology and for pharmacological or toxicological studies. First, we aimed of deriving a human skeletal muscle cell source via the efficient transdifferentiation of dermal fibroblasts into skeletal myocytes. Second, we aimed of using 3D printing to fabricate molds allowing us to assemble muscle tissues that can be assayed in real-time using fluorescent microscopy. Thirdly we aimed of combining a hydrogel component and the cell source for generating engineered skeletal muscle. Human dermal fibroblasts were transdifferentiated into skeletal muscle cells through the inducible exogenous expression of MYOD1. We utilized a combination of small molecule inhibitors, growth factors, and extracellular matrix components to significantly improve the transdifferentiation efficiency. The design of the 3D micro-mold was performed using CAD design software and the printing using a Statasys Objet260 precision 3D printer. Cells undergoing transdifferentiation were resuspended within a fibrinogen/matrigel composite hydrogel and casted within the molds. Cells were matured temporally over the span of 4 weeks. We developed a method allowing approximately 99% of the MYOD1 transduced cells to transdifferentiate into functional human skeletal myocytes. The cells expressed skeletal muscle markers, organized their cytoskeleton in a cross-striated manner and efficiently recycled Ca2+. We were able to accurately fabricate the 3D molds and determined their biocompatibility. Finally, using these micro-molds we were able to successfully bioengineer 3D skeletal muscle tissues, determine their maturation level, and characterize their functional phenotypic characteristics using genetically encoded calcium and voltage indicators. We conclude that via the efficient transdifferentiation of human dermal fibroblasts into skeletal muscle and their integration into 3D printed micro-molds we are able to reproducibly engineer human skeletal muscle that resembles in vivo human physiology.

Session No.: S40-02 Invited Speaker

A Scalable Engineering of Human Pluripotent Stem Cell Expansion and Differentiation for Clinical Application

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In recent years, cell transplantation has drawn tremendous interest as a novel approach to maintaining or even restoring function of degenerative and trauma disease. Many researchers have sought to expand and differentiate pluripotent stem cells into desired lineage cells by translating the proof-of-principle concepts into more practical and feasible protocols for scale up and manufacturing of functional cells. Here, I describe a scalable stirred-suspension bioreactor culture of human pluripotent stem cells (hPSCs) and differentiation into hepatocyte-like cells (HLCs) and cardiomyocytes. The results have demonstrated that the generated hPSCs-HLCs showed functional HLCs characteristics, improved liver function, and extended the survival of carbon tetrachloride-treated mice while enriched cells based on one of their physiological functions, the uptake of acetylated LDL-DiI, infused into their spleens. Notably, no tumor formation was detected at 15 weeks post-transplantation. Moreover, our small molecule protocol for cardiomyocyte differentiation resulted in highly efficient generation of beating cardiomyocytes with cardiac-specific gene expression and generation of action potential. This integrated approach may facilitate biomedical applications of the hPSC-derived hepatocytes and cardiomyocytes.

Session No.: S40-03 Invited Speaker

Gastric Stem Cell Culture Models for Tissue Engineering

Sherif Karam[1]

[1]Uae University

Gastric cancer is the second leading cause of cancer deaths worldwide. Treatment options include partial or total gastrectomy which has high complication and mortality rates. To use tissue engineering for repair after gastrectomy and/or mucosectomy there is a need for better understanding of the dynamics of the gastric epithelium. The stomach is lined by a single layer of epithelial cells organized to form numerous tubular glands. Using 3H-thymidine radioautography combined with electron microscopy, we have previously identified different immature epithelial progenitor cells in these glands and pinpointed the stem cells responsible for producing four main cell lineages secreting mucus, acid, pepsinogen and hormones. Alteration in the differentiation program of these stem cells in a genetically engineered mouse model led to their amplification and made it possible to establish a new epithelial cell line representative of the gastric stem cells. Growth of these stem cells in different culture systems that support their differentiation will provide the basis of developing a new therapeutic modality for treatment of post-gastrectomy syndrome.

Session No.: S40-05 Invited Speaker

Fabrication of Hydrogel Basednanocomposite Scaffold Containing Bioactive Glass Nanoparticles for Myocardialtissue Engineering

Maryam Roya Ramzgouyan[1], Jafar Ai[1], Zahra Barabadi[1], Mahmoud Azami[1]

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Selection of suitable cell source and angiogenesis induction are two important issues in myocardial tissue engineering. Human endometrial stromal cells (EnSCs) have been introduced as an abundant and easily available resource in regenerative medicine. Bioactive glass is an agent that induces angiogenesis which has been studied in some experiments. The aim of this study was to investigate in vitro differentiation capacity of endometrial stem cells into cardiomyocytelineage and to evaluate capability of bioactive glass nanoparticles toward EnSCs differentiation into endothelial lineage and angiogenesis on hydrogel scaffold. Our findings suggests that endometrial stem cells could be programmed into cardiomyocyte linage and considered a suitable cell source for myocardial regeneration, and this experiment also revealed that inclusion of bioactive glass nanoparticles in hydrogel scaffold could improve angiogenesis through differentiating of EnSCs toward endothelial lineage and increasing level of vascular endothelial growth factor secretion.
Keyword: hydrogel, nanocomposite scaffold, bioactive glass, myocardial tissue engineering

Session No.: S40-04 Invited Speaker

Theranostic Therapy Based on PEG-PCL Micelles and Folate-chitosan Nanoparticles Loading Two-photon Adsorption Fluorescence and Doxorubicin for Breast Cancer Treatment

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It has been known that breast cancer is the second leading cause of cancer death among women. In this study, a novel and advanced theranostic drug delivery system was introduced based on PEG-PCL micelles and folate-chitosan nanoparticles. The aim of this study is to prepare a nanocarrier for co-delivery of symmetrical chromophores derived from 2,3,5-trisubstituted quinoxaline units (2Q-MEH) and doxorubicin. The breakthrough of this study is to discover bio-imaging application of this novel two photon adsorption fluorescence (TPA-F) in pre-clinical cell and animal laboratory models. PEG-PCL micelles have been fabricated to successfully load TPA-F and doxorubicin. After loading, PEG-PCL micelles have been designed to be coated with folate-chitosan nanoparticles using sodium tripoly phosphate(STPP) as a cross-linker. In addition, the presence of folate on the outer surface of nanoparticles has functioned as an active targeting to cancer cells. As results, PEG-PCL copolymer (PEG5000-PCl2500, PEG5000-PCl5000, PEG5000-PCl10000) was successfully synthesized and characterized with FTIR, DSC, GPC and 1HNMR. Different molecular weight PEG-PCL micelles were fabricated and determined particles size less than 100 nm using DLS. Folate-chitosan conjugate was synthesized and characterized by FITR, DSC, UV-Vis and 1HNMR. Folate-chitosan nanoparticles were successfully prepared with particles size of less than 200 nm.

Session No.: S40-06

Peptide/Amphiphilic Polymer Dual Functionalized Graphene as a Nano-carrier for Sirna Delivery into the Breast Cancer Cells

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[3]Mcgill University

In recent years, nano-technology has revolutionized medical treatments and therapies particularly gene delivery. The development gene therapy is hindered by the absence of a safe and efficient gene carrier. Graphene, a single layer of carbon atoms in a closely packed honeycomb two-dimensional structure, is a new kind of carbon nano-structure material which attracted many attentions The aim of this study is to design methods to improve hydrocolloid stability and siRNA transfection ability of a reduced graphene oxide (rGO) based nano-carrier using a phospholipid- based amphiphilic polymer (PL-PEG) and cell penetrating peptide (CPPs). Layer-by layer graphene nano-carrier was synthesized using a non-covalent approach. The dual functionalized nano-carrier is comprehensively characterized for its chemical structure, size, surface charge and morphology as well as thermal stability. The nano-carrier cytocompatibility, siRNA condensation ability both in the presence and absence of enzyme, endosomal buffering capacity, cellular uptake and intracellular localization are also assessed. The siRNA loaded nano-carrier is used for internalization to MCF-7 cells and its gene silencing ability is compared with AllStars Hs Cell Death siRNA as a model gene. The nano-carrier remains stable in biological solution, exhibits excellent cytocompatibility, retards the siRNA migration and protects it against enzyme degradation. The buffering capacity analysis shows that incorporation of the peptide in nano- carrier structure would increase the resistance to endo/lysosomal like acidic condition (pH 6-4) The functionalized nano-carrier which is loaded with siRNA in an optimal N:P ratio presents superior internalization efficiency (82±5.1% compared to HiPerFect®), endosomal escape quality and capable of inducing cell death in MCF-7 cancer cells (51± 3.1% compared to non-treated cells). The success of siRNA-based therapy is largely dependent on the safe and efficient delivery system, therefore; the dual functionalized rGO introduced here could have a great potential to be used as a carrier for siRNA delivery with relevancy

Session No.: S40-07

Novel Gelma Hydrogel-based Blood-brain Barrier Model in Vitro

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The blood-brain barrier (BBB) controls entry of cells and molecules from the blood to the brain. The BBB regulates cell migration, water permeability, ion concentrations and transport of metabolic products to maintain neural tissues homeostasis. Endothelial cells, lining capillaries of the BBB, are characterized by enhanced tight junctions (TJ), with astrocytes playing a major role in their maintenance. Models of BBB in vitro are essential tools to investigate brain vascular diseases, drug delivery to the brain and mechanisms of cell migration across the endothelial barrier. The majority of current BBB models use composed of plastic inserts with polymer membrane at the base. The plastic inserts are placed in culture well plates growing endothelial cells on the top and astrocytes on the bottom. We are investigating a 3D co-culture system using gelatin methacrylate (GelMA) hydrogel to create a BBB model by co-culture of endothelial and astrocytic cell lines with direct contact in a GelMA hydrogel. The astrocytic cells will be encapsulated in the GelMA hydrogel allowing for 3D spatial culture mimicking the basal aspect in vivo while the endothelial cells will be cultured on the surface of the GelMA hydrogel. This novel model will be compared to common co-culture BBB models on Transwell inserts and mono culture BBB models with astrocyte cell media (ACM). The models will be characterized by transendothelial electrical resistance (TEER) as a measure of barrier tightness, Evans blue for functional tightness, trans-endothelial migration of cancer cells and immunofluorscence staining of junctional proteins expressed at the BBB.
Keywords: GelMA hydrogel, BBB, 3D cell culture, junctional complexes.

Session No.: S40-08

Effect of Different Fixation Methods on the Visco-Elastic Properties of Heart Valve Leaflets

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Two-thirds of worldwide deaths are attributable to cardiovascular diseases. Damaged and diseased heart valves constitute a significant proportion of these deaths. Aortic valve disease is the third most common cardiovascular disease. While minor damage to the heart valve could be cured by repairing the damaged area, more serious damage might render the valves beyond repair. The solution for such a scenario is surgery. Current valve surgeries replace the damaged valves with either a mechanical or a biological heart valve (porcine or bovine). However, each method of replacement presents its own challenges. Mechanical valves pose an inflammatory and thrombogenic threat. Biological valves might be rejected by the body. Natural heart valves also experience calcification causing the valve to become stiffer, leading to leakage and decreased closure during normal function. This will require the replacement of the biological valve after some years.Tissue engineering offers a viable solution to the fabrication of artificial biomimetic heart valves. Cells can be implanted onto a 3D matrix structure constructed from biocompatible and biodegradable materials. The cells grow and thrive in the structure, and then are implanted back into the body at the desired location. This process could be done to a variety of different cells and tissues, including heart valves.In this project, we study the structure of bovine heart valves and conduct rheological experiments to better understand the viscoelastic properties of heart valves, as well as the biological aspects that make them so. We vary the fixation method between paraformaldehyde, glutaraldehyde and PBS fixation. The results will help determine which fixation method least alters the physical properties of natural heart valves. Bovine heart valves are obtained from local slaughterhouses. The valves of the heart are dissected and fixed in paraformaldehyde. Rheological experiments will be carried out on the heart valves, determining their visco-elastic properties.

Session No.: S40-09

Assessing the Effect of Pomegranate and Its Active Ingredients on Neurotoxicity: Application on the in Vitro SH-SY5Y Cells and Brain Injury

Anwarul Hasan[1], Mohamad El-Moheb[2], Hisham Bahmad[2], Ola Hadadeh[2], Naify Ramadan[2], Hussein Abou-Abbass[2], Richard Younes[2], Nour Al-Rifai[2], Firas Kobeissy[2]

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Traumatic Brain Injury (TBI) is the result of a mechanical impact leading to a cascade of neural cellular injury profile. Currently, active research focuses on different approaches to ameliorate the deleterious effects of TBI using cellular, rehabilitative and drug approaches. Of interest, it has been shown that ellagic acid, the active metabolite of pomegranate juice (PJ), has neuroprotective effects in adult animal models for neurodegenerative disorders including brain injury, mediated via spectrum of cell signalling pathways to slow down the development of neural injury sequel. In our study, we will investigate the effect of PJ and its active metabolic ingredients on SH-SY5Y human neuroblastoma cell line under the apoptotic (through Staurosporine – STS), necrotic (through Maitotoxin – MTX), and excitotoxic (through N-Methyl-D-Aspartate – NMDA) challenges. In addition, the work will be extended to in vivo mouse model of experimental TBI (controlled cortical impact CCI model) where animals will be provided free access to PJ and different endpoints will be assessed including behavioural, cellular, as well as pathological ones.

Session No.: S40-10

Silk/Swnt/Fibronectin Nerve Guide Conduit for Peripheral Nerve Regeneration

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As a contribution to the functionality of nerve guide conduits (NGCs) in nerve tissue engineering, here we report a conduit processing technique through introduction and evaluation of topographical, physical and chemical cues. Porous structure of NGCs based on freeze-dried silk fibroin/single walled carbon nanotubes (SF/SWNTs) has shown a uniform chemical and physical structure with suitable electrical conductivity. Moreover, fibronectin (FN) containing nanofibers within the structure of SF/SWNT conduits produced through electrospinning process have shown aligned fashion with appropriate porosity and diameter. Moreover, fibronectin remained its bioactivity and influenced the adhesion and growth of U373 cell lines. The conduits were then implanted to 10 mm left sciatic nerve defects in rats. The histological assessment has shown that nerve regeneration has taken places in proximal region of implanted nerve after 5 weeks following surgery. Furthermore, nerve conduction velocities (NCV) and more myelinated axons were observed in SF/SWNT and SF/SWNT/FN groups after 5 weeks post implantation, indicating a functional recovery for the injured nerves. With immunohistochemistry, the higher S-100 expression of Schwann cells in SF/SWNT/FN conduits in comparison to other groups was confirmed. In conclusion, an oriented conduit of biocompatible SF/SWNT/FN has been fabricated with acceptable structure that is particularly applicable in nerve grafts. Key words: Nerve guide conduit, Silk fibroin, Single walled carbon nanotubes, Fibronectin, U373 cell line.

Session No.: S40-11

A Tissue Engineered Heart Valve Leaflet Using PCL-PLLA Scaffolds

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Heart valve diseases are among the major causes of mortality around the world. Currently bioprosthetic or mechanical heart valves are among the standard substitutes for diseased native heart valves. These replacements represent several deficiencies such as they are unable to grow, self-repair, or remodel. Moreover, the biological valve-substitutes have a life expectancy of only 10-15 years while the mechanical valves are thrombogenic which requires life-long anti-coagulation therapy. Tissue engineered heart valves are promising alternates to overcome these drawbacks. They will enable the valves to maintain their biochemical and mechanical properties, as well as grow with the patient. In this study, we report the development of electrospun poly(e-caprolactone) (PCL)-poly(L-lactic acid) (PLLA), scaffolds of different concentrations and compositionsfor their application in tissue engineering of heart valve leaflets. Characterization of these scaffolds were performed through scanning electron microscopy (SEM), X-Ray Diffraction (XRD), and uniaxial tensile mechanical testing. The biological characterization of the produced scaffold were tested using seeding of cardiac stem cells (CSC) and endothelial cells and performing MTT assay and the immunofluorescence microscopy. Experimental results revealed that the combination of PCL and PLLA strengthened the scaffolds, made it more pliable at certain specific compositions. Also, biological response demonstrated that the developed scaffolds were cytocompatible and showed a significant adhesion and proliferation of seeded cells onto the constructs. These results indicated that the blends of PLLA and PCL are promising materials for applications in heart valve tissue engineering.

Session No.: S41-01 Keynote Speaker

Scaffolds with TGF-β1 Affinity Promoted Chondrogenesis of BMSCs and Cartilage Regeneration

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Introduction: TGF-beta plays a critical role in chondrogenesis [1]. The concentration of TGF-beta is crucial for chondrogenic differentiation. In our study, porous chitosan scaffolds were carried out, modified by peptides with affinity to TGF beta1 which could retain the bioactivity of TGF-beta and control TGF-beta release in spatial regulation. We aim to develop a strategy to regenerate cartilage in situ defect by only introducing this scaffolds without addition with extra growth factors and cells. Materials & Methods: Briefly, the chitosan sponge was fabricated using a previously described method [2]. TGF-beta affinity peptide was cross-linked to the chitosan sponge using EDC/NHS method. Chitosan sponges with peptides were prepared as the experimental group, chitosan (without peptide) served as the control group. Cell skeleton staining and gene expression were carried out to detect the effect of TGF-beta affinity sponge, then we used nude mice model and rabbit cartilage defect model to confirm the cartilage formation ability in ex-situ and in-situ. Results & Discussion: The gene expression of collagen II, aggrecan and Sox 9 were increased by approximately 2, 4, 3 folds in the peptide crosslinked chitosan scaffolds. For in vivo cell skeleton staining, F-actin fiber arranged firmly surrounding cell clusters to form spherical morphologies, but for the control group, F-actin fiber change to a multilateral shape. The specific immunostaining performed confirmed that the construct composed of TGF-beta 1 affinity peptides evidently enhanced the accumulation and homogeneity of collagen II compared to the control group in both nude mice for 4 weeks and rabbit model for 6 months. Conclusions: In conclusion, TGF-β1 affinity peptides crosslinked scaffolds maintained the spherical morphologies of the cells and promote the synthesis and expression of the cartilage matrix both in vitro and in vivo. Key words: TGF-β, cartilage, mesenchymal stem cells References: [1].van der Kraan, P.M., et al., TGF-beta signaling

Session No.: S41-02 Invited Speaker

Novel Bilayered Artificial Dermis with Sustained Release of Growth Factors: Clinical Trial for Chronic Ulcers

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A bilayered artificial dermis, composed of an upper silicone sheet and a lower collagen sponge, has been used clinically since 1990's. After application, fibroblasts and capillaries penetrate and proliferate in the collagen sponge and host dermis-like tissue is formed according to the biodegradation of the collagen sponge. Chronic skin ulcers such as diabetic ulcers and venous leg ulcers are increasing and are a costly problem inhealthcare. We have developed a novel artificial dermis, collagen/gelatin sponge (CGS), which is capable of sustained release of basic fibroblast growth factor (bFGF) for more than 10 days. The objective of this study was to investigate the safety and efficacy of CGS impregnated with bFGF in the treatment of chronic skin ulcers. Patients with chronic skin ulcers that had not healed in at least 4 weeks were treated with CGS impregnated with bFGF at 7 or 14 mg/cm2 after debridement, and the wound bed improvement was assessed 14 days after application. Wound bed improvement was defined as a granulated and epithelialized area on day 14 with a proportion to the baseline wound area after debridement of 50% or higher. The wound area, the wound area on day 14, and the granulation area on day 14 were independently measured by blinded reviewers. Patients were followed up until 28 days after application to observe the adverse reactions related to the application of CGS. 17 patients were enrolled and, in 16 patients, the wound bed improved. Adverse reactions with a clear causal relationship to the study treatment were mild and patients quickly recovered from them. This study is the first-in-man clinical trial of CGS and showed the safety and efficacy of CGS impregnated with bFGF in the treatment of chronic skin ulcers. This combination therapy could be a promising therapy for chronic skin ulcers.

Session No.: S41-03 Invited Speaker

Design of Fc Fusion Proteins for Maintenance and Differentiation of Pluripotent Stem Cells

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To regulate cell function in vitro, various biomaterials have been generated. We have focused on recombinant protein-based biomaterials and designed several fusion proteins that consist of a cell-recognizable domain and an Fc domain from immunoglobulin G as an immobilizable domain. E-cad-Fc, which is a fusion protein combining the extracellular domain of E-cadherin with the Fc region, was generated to convert a cell-cell adhesion into a cell-substrate interaction. We have applied E-cad-Fc to the establishment of a novel culture system designed to maintain pluripotent stem cells. Mouse ES cells cultured on conventional gelatin-coated plate or on a feeder layer of mouse embryonic fibroblasts form tightly aggregated colonies. On the other hand, mouse ES cells did not form colonies on E-cad-Fc surface; cells were scattered around each other without any cell-cell interaction. Mouse ES cells retained undifferentiated state and germ-line transmission ability, although they showed scattering behavior. In contrast to mouse ES cells, human pluripotent stem cells (hPSCs) cultured on the E-cad-Fc-coated surface formed colonies similar to those formed on feeder cells or on the Matrigel surface. hPSCs cultured on the E-cad-Fc-coated surface could generate all three germ layer-derived tissues by forming teratomas. These results revealed that mouse and human ES/iPS cells could be maintained under completely defined conditions using E-cad-Fc as a culture substrate. For the differentiation of hPSCs, a highly defined cell adhesion material, R-Fc, which includes a small fragment of vitronectin containing the RGD motif, was generated. This protein can be used to both maintain the pluripotent state of hPSCs and differentiate them into hepatocyte–like cells. The design of suitable materials for specific cell types could improve the maintenance of undifferentiated stem cells and also controlled differentiation from stem cells to cells of target lineage under completely defined condition that eliminate xenogeneic components.

Session No.: S41-04

Migration Behavior of Endothelial Cells in 3D Biomaterials to Mimic the Repair of Blood Vessels in Vivo

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Keywords: endothelial cells, collagen-chitosan scaffolds, macrophages, 3D migration Abstract: Cell migration plays a crucial role in a variety of physiological and pathological processes ranging from wound healing, revascularization, immune response, tumor metastasis, embryonic development and so on[1]. When a signal gradient exists, the imbalanced force exerted on the cells leads to cell membrane and cytoskeletal polarization. At that time, the cell ends are rendered differently, so as to guide the directional movement of cells. Cell migration consists of two-dimensional and three-dimensional migration. Most of the experiments have been based on planar migration [2]. However, compared with two-dimensional cell migration, the three-dimensional migration can better simulate morphology of cells in vivo and provide the theoretical knowledge about the repair of blood vessels. In this study, a kind of initial model based on Transwell was proposed. Phenotypes of macrophages were polarized by 50 ng/ml interferon-gamma and different contents of lipopolysaccharide (150-300ng/mL) to produce vascular endothelial growth factor as the chemo-attractant for endothelial cells. Using Transwell model, cell invasion through the collagen-chitosan scaffolds was observed to mimic the cell migration during blood vessel repair in vivo. The results show that as the concentration of lipopolysaccharide increased, cells could migrate obvious deeper into the scaffolds compared with inactivated macrophages measured by the confocal laser scanning microscopy. More importantly, the endothelial cells could migrate deeper into the scaffolds with large pores than that with small pores fabricated by freeze-drying under different temperatures. This research proposes a useful strategy to promote the invasion and proliferation of endothelial cells into a scaffold combined with the macrophage induction, which may be further applied to the cardiovascular therapeutics. Acknowledgements This study is financially supported by the Natural Science Foundation of China (21434006, 21374097). References: [1] Ridley AJ, et al. Cell migration: Integrating. Science 2003;302:1704-9. [2] Gao CY, et al. Biomaterials 2015;56:58-67.

Session No.: S41-05

Heparin-immobilized Thermoresponsive Surface for Manipulating Hepatocyte Sheets with Maintenance of Hepatic Functions

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Liver tissue engineering using primary hepatocytes is an attractive method for the treatment of liver diseases. Engineered Hepatocyte sheets were effectively engrafted in pre-vascularized subcutaneous site and exhibited liver-specific functionalities. By contrast, cultured hepatocytes rapidly lose their viability and phenotypic functions on isolation from the native in vivo microenvironment of the liver. To overcome this problem, heparin-immobilized poly(N-isopropylacrylamide) (PIPAAm)-grafted cell culture surface, which interacts with heparin-binding proteins such as heparin-binding EGF-like growth factor (HB-EGF), was been designed for maintaining hepatic functions during the cultivation. In addition, the detachment of the cultured hepatocytes as a sheet was examined when lowering temperature to 20 ℃. The addition of soluble HB-EGF in the cell culture media was essential for the survival of hepatocytes. When the medium contained less than 10 ng/cm^2 of soluble HB-EGF, the hepatocytes were not able to adhere and form their cell sheets. By contrast, hepatocytes adhered and formed their sheets on heparin-immobilized thermoresponsive surface with 10 ng/cm^2 of bound HB-EGF. In addition, the secretion of albumin on bound HB-EGF was maintained and higher than that on PIPAAm-grafted surfaces with soluble HB-EGF. Therefore, bound HB-EGF gave a high activity of maintenance of hepatocyte adhesion and function compared with soluble HB-EGF. Finally, when lowering temperature to 20 ℃, the cultured hepatocyte sheets were detached from the surface through the reduction of affinity binding between HE-EGF and immobilized heparin with increasing the mobility of heparin and steric hindrance of the swollen PIPAAm chains. In conclusion, heparin-immobilized thermoresponsive cell culture surfaces facilitated the manipulation of hepatic cell sheets with maintaining hepatic functions by changing temperature. Creation of transferable and functional liver tissues is considered to have a potential to treat liver disease.

Session No.: S41-06

Mechanochemical Regulation of Macrophages by Engineered Biopolymer Scaffolds

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Macrophages are essential regulators of the innate immune system, and play an important role in advancing and resolving inflammation during wound healing. To perform their functionally diverse roles, these cells can rapidly change their function in response to cues in their surrounding microenvironment. For example, inflammatory stimuli such as interferon-gamma and toll like receptor ligands lead to macrophage polarization toward a classically activated phenotype and production of cytokines and reactive species to promote inflammatory signaling. However, exposure to a wound healing environment causes the same cells to polarize toward an alternatively activated phenotype, which promotes tissue healing and regeneration. While much is known about how soluble cues in the environment regulate macrophage phenotype, less is understood about how physical cues modulate their behavior. In particular, the effects of changes in adhesive environment caused by matrix remodeling during wound healing have not been clearly elucidated. Our laboratory recently showed that the geometry of cell adhesion plays an important role in macrophage polarization; specifically cell elongation induced by micropatterned substrates promotes the expression of markers associated with an alternatively activated, pro-healing phenotype. In current work, we investigate how the composition and structure of three-dimensional fibrillar matrices regulate macrophage cell shape and function. We developed three-dimensional matrices composed of natural biopolymers including collagen and fibrin, which are present at varying concentrations and proportions during the wound healing process. While macrophages remained mostly rounded when seeded on three-dimensional matrices, the presence of fibrin enhanced cell spreading and the formation of cell protrusions into the matrix. In addition, our studies suggest that the mechanical tethering of matrix molecules regulates a switch between inflammatory and anti-inflammatory behavior of macrophages. A better understanding of how physical and adhesive cues regulate macrophage behavior will ultimately aid in the design of biopolymer scaffolds for tissue engineering and regeneration.

Session No.: S41-07

Nagelschmidtite Bioceramics Regulates Osteoblast Differentiation Through BMP2 Signalling Pathway

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The aim of this study was to investigate the interaction of MC3T3-E1 osteoblasts with nagelschmidtite (NAGEL, Ca7Si2P2O16) bioceramics ionic extracts, and to explore the possible molecular mechanism. Firstly, we investigated the biological effects of ionic products from NAGEL powders on osteoblast migration. NAGEL extracts significantly induced migration of MC3T3-E1 cells in both a Boyden chamber assay and in wound healing assay in a dose-dependent manner. In the in vitro differentiation assay, mineralization of MC3T3-E1 osteoblasts was demonstrated by Alizarin red staining assay and ALP activity assay after NAGEL extracts treatment. These findings indicate that NAGEL induces osteogenic differentiation in MC3T3-E1 osteoblasts. It is known that bone morphogenetic proteins (BMPs), especially BMP2, are crucial regulators of osteogenesis. However, the relationship between NAGEL extracts-mediated osteogenesis and BMP2 is unclear. We found that NAGEL extracts significantly enhances mRNA expression of BMP2 in a dose-dependent manner. Moreover, NAGEL extracts-induced osteoblast proliferation and migration activity were significantly decreased after treatment with neutralizing BMP2 antibodies and BMP2 inhibitor Noggin. Further evidence showed that NAGEL extracts-induced bone mineralization was inhibited by neutralizing BMP2 antibodies in a dose-dependent manner. In an ALP activity assay, both BMP2 neutralizing antibody and Noggin inhibited NAGEL extracts-induced ALP activity in osteoblast cells. These findings indicate that BMP2 is critical for NAGEL extracts-mediated osteoblastic differentiation.

Session No.: S42-01 Keynote Speaker

Cellular Delivery of Therapeutics to Tumor Hypoxia

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Hypoxic regions frequently develop in malignant solid tumor due to rapid tumor growth. These areas are characterized with the absence of tumor blood vessels. Therefore, conventional therapeutic delivery approaches via systemic blood circulation to tumor hypoxia are severely restricted. Two approaches presented in this study demonstrate the potential of monocyte-mediated delivery for improving chemotherapy and photodynamic therapy on tumor hypoxia. The bone marrow-derived monocytes capable of being chemotactically recruited to tumor hypoxic regions were employed as a cellular Trojan for active targeted delivery of therapeutics toward tumor avascular regions. In cell-based chemotherapy, successful cellular transport of nanotherapeutics to tumor hypoxic regions and pronounced chemotherapeutic action against hypoxic cancer cells through the remote-controlled focused ultrasound-triggered drug liberation from cellular hosts in vivo was achieved. The chemotactic behavior of therapeutic monocytes towards tumor hypoxia also gave rise to the highly enhanced chemotherapy efficacy on the tumor-bearing mice pretreated with gamma-irradiation, known as an external energy to disrupt blood supply to tumors and thus reduce therapeutic transport via blood circulation. In cell-based photodynamic therapy, the tumortropic monocytes laden with oxygen/phototherapeutics payloads were exploited for enhancing efficacy of photodynamic therapy in tumor hypoxia. The IHC examinations of tumor sections showed the pronounced antitumor effect of the photodynamic therapy along with additional oxygen supply in tumor avascular regions. The results demonstrate the great promise in the monocytic delivery of therapeutic payloads to improve therapeutic efficacy in tumor hypoxia.

Session No.: S42-02 Invited Speaker

Cationic Polymer-coated Superparamagnetic Iron Oxide Nanoparticles as an Efficient Magneto-gene Carrier

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Designing a gene drug delivery system with low cytotoxicity and high therapeutic efficacy of drugs is currently a challenging task. For delivering nucleic acids, cationic polymers such as polyethylene imine (PEI) and poly(2-dimethylaminoethyl methacrylate) (PDMAEMA) are the major type of non-viral gene delivery vectors. We will introduce several synthetic methodologies to improve transfection efficiency as well as to reduce cytotoxicity. For example, we combined a natural polysaccharide, chondroitin sulfate (CS), with PEI to see the remarkably-reduced inherent cytotoxicities of nascent polymers. The good uptake of CS-modified polymers/pDNA into cells is due to facilitating CD44-mediated endocytosis. We also use a “Magnetofection” technique to enhance the gene expression. Binding PDMAEMA onto superparamagnetic iron oxide nanoparticles (SPION) surface was developed using atom transfer radical polymerization (ATRP). The PDMAEMA@SPION/pDNA magnetoplex exhibited remarkably improved gene expression in both the presence of a magnetic field and 10% FBS as compared with a commercial product, PolyMag/pDNA.

Session No.: S42-03 Invited Speaker

Microfluidics-controlled Synthesis of Functional Nanomaterials for Drug Delivery

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Hybrid core-shell nanoparticles are becoming a new class of drug nanocarriers due to for their improved therapeutic efficacy and enhanced biocompatibility. We present a hollow-structured rigid nanoparticle fabricated by a multi-stage microfluidic chip in one step, to effectively entrap various hydrophilic reagents inside, without complicated synthesis, extensive use of emulsifiers and stabilizers, and laborious purification procedures. The nanoparticle contains a hollow water core, a rigid poly (lactic-co-glycolic acid) (PLGA) shell, and an outermost lipid layer. The entrapment efficiency of hydrophilic reagents such as calcein, rhodamine B and siRNA inside hollow water core is ~ 90 %. In comparison with the combination of free Dox and siRNA, the hollow-structured rigid nanoparticle that co-encapsulates siRNA and doxorubicin (Dox) reveals a significantly enhanced anti-tumor effect for a multi-drug resistant tumor model. Lipid-covered PLGA NPs or liposomes of the same size and surface properties, but different rigidity by controlling the interfacial water layer, can be realized using a two-stage microfluidic chip. It enables us to explore how rigidity of NPs regulates the cellular uptake and elucidate the intrinsic mechanism. Given the only significant difference between those two types of NPs is the rigidity, the experiments suggest that rigidity could dramatically alter the cellular uptake efficiency, with rigid NPs being easier to get through members than soft ones. The mechanisms revealed here suggest that tuning of rigidity of NPs is one appealing way in improving therapeutic efficiency.

Session No.: S42-04 Invited Speaker

Fe-Au Nanomaterials for Theranostic

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[1]National Taipei University of Technology (Taipei Tech)

The aim of this study is to develop Iron-Gold Alloyed nanoparticles (NFAs), which are superparamagnetic and of great potential to be applied in the biomedical fields, including hyperthermia treatment for cancer and drug controlling release. The NFAs were prepared through a pyrolysis method and well dispersed in distilled water. The ratio of iron to gold was 2 : 1 through analyzing. The mean diameter was 3.932 nm. The saturated magnetization was 3.5 emu/g under 20 KOe magnetic field. The material was superparamagnetic at room temperature. The result of in vitro tests showed that under a dose of 500μg/mL were not cytotoxic to L929 cell and Hep-G2 cell. Methotrexate (MTX) is an anti-cancer medicine and able to target the cancer cells. MTX was further conjugated on to NFA through a series of chemical modifications, including 2-aminoethanethiol grafting, and then the amine bonded with the carboxyl group on MTX to form an amide bond. Immobilization of MTX on the NFA was confirmed using FTIR. The uptake of Hep-G2 cells was 0.789 pg/cell for NFA-MTX, which was 1.5 times of the L929 cells. The results indicated the targeting efficiency of NFA-MTX to cancer cells. After calculation, per NFA would generate a 2.03×10-16 J heat under a high frequency magnetic field (700 to 1100)KHz. After treating with 200μg/mL NFA-MTX and then being applied a (700 to 1100) KHz high frequency magnetic field treatment for 20 minutes, the residual viability of Hep-G2 cells dramatically decreased 45%. Besides, we also discovered that the releasing of MTX from NFA-MTX was dependent with the applied time of high frequency magnetic field. The generated heat was able to break the amide bonds between NFA and MTX. The release of MTX could be successfully controlled.

Session No.: S42-05

Lysozyme Loading and Release from Selenium-doped Hydroxyapatite Nanoparticles

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Nanocomposites consisted of element-doped hydroxyapatite (HA) have received more and more attention as promising therapeutic materials for improving bone defect repair. Selenium-substituted HA (Se-HA) nanoparticles can not only induce the apoptosis of bone tumor cells, but also tremendously enhance osteointegration. However, the effect of selenite ions on the proteins in combination with the HA nanoparticles remains not clear. In this study, we investigated the influence of selenium doping concentration on the loading and release of lysozyme (LSM) as a model protein drug. Se-HA/LSM composites with different selenium concentrations were synthesized and characterized, respectively. The subsequent delivery of LSM was studied in a phosphate buffer solution (PBS). We found that Se-HA/LSM composites with the 10% ratio of Se to P showed the highest amount of LSM loading (41.7%) whereas the amount of lysozyme loaded in pure HA nanoparticles was the lowest (34.1%). The doped selenium interacted with lysozyme molecules leaded to the increase of β-sheet and unordered, and the decrease of self-association, α-helix and β-turns in protein secondary structures. Moreover, the addition of selenium significantly declined the protein release from Se-HA/LSM composites. The composites with the 10% ratio of Se to P release lysozyme at the slightly slower rate among the samples with different Se doping concentrations. More important, the released LSM still maintains its enzymatic activity. In consequence, our current study paves a new way to fabricate a self-therapeutic nanoparticle of Se-HA with dual function on directing the apoptosis of tumor cells and promoting bone repair and regeneration.

Session No.: S42-06

Carbon Nanotube Activate Amebocyte Lysate

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Endotoxin test is requested by regulatory authorities of USA and China for assessing endotoxin contamination in products for human use. Engineered nanoparticles are easily contaminated by bacterial endotoxin, a ubiquitous bacterial molecule with significant toxic and inflammatory effects. The presence of endotoxin, if not be recognized, can be responsible for many of the in vitro and in vivo effects that would lead to misunderstanding of nanoparticles performance. In this work, oxidized multiwalled carbon nanotube (Os-MWCNT) that can endure high temperature treatment and be well dispersed in water with high concentration, was prepared. By heated up to 180 ℃ for 4 h, exogenous endotoxin was completely eliminated. Next we investigated the interaction of carbon nanotube to Limulus Amoebocyte Lysate (LAL) assay. Our results showed that the Os-MWCNT dispersing in the aqueous solution induced LAL positive reaction (gel formation) even at 10 ng/mL. The reaction between Os-MWCNT and LAL was independent on the β-1,3-D-glucans (BDG)-mediated pathway. The Os-MWCNT induced a concentration dependent positive reaction in the chromogenic LAL assay. Results obtained from the chromogenic LAL assay were consistent with that from the gel clot assay. Results of spiking experiments with the gel clot LAL showed enhanced effects elicited by Os-MWCNT. The Os-MWCNT dispersing in the aqueous solution containing bovine serum showed a significantly lower “endotoxin” like responses comparing to that of Os-MWCNTs dispersing in water. It is reasonable to consider that the serum protein molecules adsorbed on Os-MWCNT changed its surface property or the serum protein molecules in the aqueous solution interfered the interaction between LAL and Os-MWCNT directly. In conclusion, when dispersing in the aqueous solution, Os-MWCNT could induce LAL assay false positive reactions and interfered with the LAL test.

Session No.: S43-01 Keynote Speaker

In Vivo Models for Functional Cartilage and Bone Tissue Engineering

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[1]University of Leeds

The development and choice of an appropriate model system is crucial for tissue engineering and the restoration of normal tissue/organ structure and function. Many different in vitro models have been developed and used to meet this target, such as spheroid culture, pellet culture, 3D scaffold construct, 3D cell printing and bioreactors. However in vitro models are often unable to fully mimic the appropriate physiologically-relevant micro-environment and hence animal models are an essential pre-requisite in the translation of any new therapy to the clinic. To date, in vivo animal models are still considered as the gold standard for testing the capacity of stem/progenitor cells, smart biomaterials and novel growth factors for successful bone and cartilage tissue engineering. Ideal animal models should mimic clinically relevant conditions and provide an appropriate permissive microenvironment (e.g. angiogenesis, mechanical stimuli and physiological/pathological conditions) for tissue regeneration. Over the last decade, eight different animal models have been developed/adopted to support our research on skeletal tissue engineering. The key factors driving the choice of a given animal model will depend on the requirements of the experimental design, the hypothesis and thespecific parameters to be tested. In this talk, the choice of animal (include species, age and size) and the different animal models commonly used for bone and cartilage tissue engineering, together with their respective advantages and limitations will be discussed; this will range from relatively simple experimental designs, such as the subcutaneous/intramuscular implant models, through to the diffusion chamber model and chorioallantoic membrane (CAM) assay, to the more complex in vivo bioreactors, clinically relevant bone, cartilage and osteochondral defect models. The need to consider the ethical issues of using animal models and the principles of reduction, replacement and refinement will also be emphasised. Acknowledgement: WUN and EU FP7 under grant agreement no [318553] - SkelGEN.

Session No.: S43-06 Keynote Speaker

Modular Inductive High-density Cell Culture Systems for Engineering Complex Tissues

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High-density cultures of cells can mimic immature condensates present during many developmental processes. Presenting specific soluble signals, such as growth factors, exogenously in tissue culture media can regulate cell behavior in these cultures and promote new tissue formation. However, shortcomings of this approach include transport issues, limited spatial control over signal presentation, and required repeated dosing in the media. We have engineered technology that overcomes these challenges by incorporating polymer microspheres containing bioactive signals within the high-density cell cultures, which permits localized spatial and temporal control over the presentation of these regulatory signals to the cells. In this talk, I will present our research using this strategy to engineer a variety of tissues, including bone, cartilage and trachea. The capacity to deliver diverse signals, including growth factors and plasmid DNA, for driving new tissue formation will be demonstrated. In addition, the value of this technology for engineering a wide range of tissue shapes, including spheres, sheets, rings and tubes will be shown. Finally, the utility of providing cell-instructive bioactive factors from biomaterials in a controlled manner for the assembly of modular tissue units to engineer complex constructs comprised of multiple tissue types will be explored.

Session No.: S43-02 Invited Speaker

In Vitro Fabrication of 3D-engineered Tissues for Organ on a Chip

Michiya Matsusaki[1], Mitsuru Akashi[1]

[1]Osaka University

In vitro development of highly-organized three dimensional (3D)-engineered tissues consist of multiple types of cells and ECM, which possess a similar structure and function as natural tissues, is a key challenge for tissue engineering and pharmaceutical assay. Especially, "organ on a chip" is the current hot topic as a next generation pharmaceutical technology. Human organ on a chip integrating 3D-engineered tissues will be a powerful technology altering an animal experiment. We have developed a simple and unique bottom-up approach, "hierarchical cell manipulation", using nanometer-sized Layer-by-Layer films consisting of fibronectin and gelatin (FN-G) as a nano-extracellular matrix (nano-ECM). The FN-G nanofilms were prepared directly on the cell surface, and we discovered that at least 6 nm thick FN-G films acted as a stable adhesive surface for adhesion of the second cell layer. We have also developed a rapid bottom-up approach, "cell-accumulation technique", by a single cell coating using FN-G nanofilms, because the fabrication of two-layers (2L) was limitation through the above technique due to the time required for stable cell adhesion. This rapid approach easily provided more than twenty-layered 3D-tissues after only one day of incubation. Moreover, fully and homogeneously vascularized tissues of 1 cm width and over 100 µm height were obtained by a sandwich culture of the endothelial cells. Since these technologies easily applied to cell-inkjet printing system, both manipulations will be promising to achieve organ on a chip.

Session No.: S43-03 Invited Speaker

A New Physiologically-relevant Liver Tissue Model Based on Hierarchical Coculture Using Oxygen-permeable Multiwell Plates

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In vivo hepatic microenvironment taked a complex structure comprising multiple cell types and their better in vitro organization in very important. Although cocultures havc reported to gerenally enhance the functions, there are only a few systems that enable in vivo-like hyerarchical structures such as complete cell layers of heopatocytes and sinusoidal endothelial cells. We here report multiwell plate-based simple assembly of a novel organotypic liver model incorporating rat hepatocytes and liver sinusoidal endothelial cells (TMNK-1) using oxygen-permeable polydimethylsiloxane (PDMS) plates. The bottom PDMS membranes could facilitate direct oxygenation by diffusion through the membrane, solving the problem of oxygen shortage encountered in the conventional tissue culture-treated polystyrene (TCPS) in static culture. Complete double layers of hepatocytes and TMNK-1 cells was simply obtained by overlaying TMNK-1 cells onto preformed confluent hepatocyte layers, on the collagen-crosslinked PDMS membranes. In contrast in TCPS, both two cell populations tried to occupy the same culture surfaces and finally hepatocytes formed a island-like structures surrounded by TMNK-1 cells on the bottom surfaces. Such complete double layered structure was well-maintained for at least 14 days. Vertical and intimate contacts of the tew cell poplulations in individual cell-cell contacts remarkably promoted the hepatocytes to exhibit cuboidal morphology. The hepatocytes also exhibited improved metabolic activities such as albumin production and CYP1A1/2 activity, and functional bile canaliculi formation and polarization as evidenced better transporter expressions. Another advantages of PDMS plates are that it enables in vivo-like aerobic respiration without perfusing culture mediums and that we may easily include other cell populations such as stellate cells or Kupfer cells. These results demonstrate the new coculture using PDMS plates can serve as a simple and better physiologivally-relevant organotypic hepatic model in various in vitro applcations such as not only tests for efficaly/toxicity of chemicals but also as in vitro disease models.

Session No.: S43-04 Invited Speaker

A Miniature, Tissue-Engineered Lung - PuLMo

Jen-Huang Huang[1], Pulak Nath[2], Ayesha Arefin[2], Kirill Balatsky[2], Yulin Shou[2], Jennifer Harris[2], Rashi Iyer[2]

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Drug discovered through animal testing have been proven to be inefficient in many instances.. Even some pharmaceuticals that successfully pass clinical trials are later found to have serious side effects that can lead to unwanted suffering, costly lawsuits, or even worse, the death of patients. The current technology developed to emulate organ level functions in miniaturized tissue-engineered models is known as “micro-physiological systems” or “organs-on-a-chip”. These models have been used to study the adsorption, distribution, metabolism, elimination, and toxicity (ADMET) of drugs in vitro. However, until now there are only few such systems that can integrate both biological and biophysical features to recapitulate a complex and functional human lung. To capture multiple critical features of the human lung system in vitro, we have undertaken a stepwise approach to engineer a multilayered microfluidic platform called PuLMo (Pulmonary Lung Model) that integrates both bronchiolar and alveolar features. PuLMo was designed to co-culture at least three different cell types from three different regions of the lung: Bronchiolar Epithelial cells, Alveolar Epithelial cells, and Microvascular cells. It also incorporates several physiological characteristics such as air-liquid interface (ALI), ciliated cells, mucus production, cyclic stretching of membranes, surfactant production, shear flow on microvascular cells, and breathing. Breathing motion is carried out using a novel, non-pneumatic microfluidic aspiration mechanics. Complete operation of PuLMo requires management of at least five different media; frequent transition between liquid-liquid interface and air-liquid interface; and mucus clearance. To enable the complex flow management scheme for fully automated and semi-automated operations, novel pumps, valves, fluid circuit boards, and reservoirs were also developed and integrated with PuLMo.

Session No.: S43-07 Invited Speaker

In Vitro Tunable Cell and Tissue Culture System for Understanding Salivary Gland Tissue Development

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[1]Okayama University

Xerostomia is often a consequence of autoimmune disease, radiation therapy or aging, and is related to dysphagia, aspiration pneumonia and dysarthria, which strongly impair the individual's life activities. Therefore, it is crucial to understand salivary gland tissue development for developing new treatment methods for such diseases. In this context, recent advancements in in vitro cell and tissue manipulation techniques are valuable to obtain new insights into salivary gland development. We have recently developed a hydrogel culture system as well as a salivary gland tissue synthesis model that enable modulation of salivary gland tissue growth in vitro with physical and/or chemical stimulation. By using these two in vitro culture systems, we investigated the presence of macrophages and the effect of macrophage colony stimulating factor (MCSF), a key regulator of macrophage differentiation, on salivary gland branching morphogenesis. Interestingly, we found that MCSF is one of the key factors for salivary gland tissue development, by regulating FGF-7 and FGF-10 expression and neuronal network development. We also applied the in vitro tissue synthesis model to confirm the effects of MCSF on the early stages of epithelial bud formation, and found that blockade of MCSF with specific antibody attenuates salivary gland development and neuronal innervation. These in vitro model systems enable easy and precise tuning of experimental conditions and tissue culture environment; thus it would be crucial for the next stage of life science researches, and possibly for the development of novel treatment modalities for xerostomia.

Session No.: S43-05

A Novel 3Dp Bioceramic Bone Scaffold with Optimal Mechanical Strength and Cell Affinity

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Artificial bone grafting is widely used in current orthopedic surgery for bone defect problems. Unfortunately, surgeons remain unsatisfied with the current commercially available products. The repair or replacement of injured or defective bone is a critical problem for orthopedic surgeons. Recently, silica bioceramics have been widely used for bone restoration and bone tissue engineering, but silica is insufficient to support the bone structure. In this study, we add CaCO3 into slurry to enhance mechanical property by laser-aided gelling (LAG) method on a self-developed 3D printer. The principal materials used in this study were SiO2 powder. The SiO2 sol and SiO2 powder were mixed at a 20/80 ratio (w/w) to produce a SiO2 slurry, termed CS0. CaCO3 was another powder additive that served as a filler substance and solid content after sintering. CaCO3 powder was added separately to the SiO2 slurry at weight ratios of 5% and 9%, termed CS5 and CS9, respectively. In order to assess the scaffold feasibility by compressive test, SEM, XRD and MTT assay. The LAG method and a home-made 3DP machine were used to produce bioceramic bone scaffold. The maximum compressive strength of CS5 was 47 MPa and the porosity was increased to 34%. The optimum CS5 scaffold shows no cytotoxicity and good bone cell attachment and growth. The inter-porous silica bioceramic scaffolds with a pore size of 0.8 mm has been successfully fabricated.

Session No.: S44-01 Keynote Speaker

Bioinspired Synthetic Inorganic Nanoparticles for Bone Tissue Engineering

Nathaniel Hwang[1], Hwan Kim[1], Hae Lin Jang[1], Ki Tae Nam[1]

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Recently, synthetic matrices emulating the physiochemical properties of bone tissues are being developed to control stem cell fate. Biomaterials containing calcium phosphate moieties, such as hydroxyapatite (HAP: Ca10(PO4)6(OH)2), have been shown to induce osteogenic differentiation of stem/progenitor cells and bone tissue formation. Recent evidences suggest that one of the main components of inorganic phase of bone is whitlockite (WH: Ca18Mg2(HPO4)2(PO4)12). Even though WH a relatively rare mineral in nature, it is the second most abundant mineral in human bone with approximately upto 20 wt%, and it is particular found in bone with elevated dynamic loading. The increased detection of short micro-ranged WH in bone under increased loading along with its acidic stability suggests that it may act as an inorganic template composition for further mineralization. In addition, elevated composition of WH in adolescent's bone suggest that it may be actively involved in bone remodeling process. Here, we investigated the role of WH on bone formation. WH nanoparticles were synthesized, and we demonstrate that the synthetic WH can recapitulate early-stage bone regeneration via elevated extracellular PO43- and Mg2+ concentration. Furthermore, our studies showed that WH participates in bone formation via increased affinity with extracellular proteins. Finally, we demonstrated that the WH containing scaffold platform could stimulate in vivo bone formation. The multidisciplinary approach conducted in this study provided an organized methodology to find biological functionalities of WH and introduced a useful clinical application of WH for bone formation.

Session No.: S44-02 Invited Speaker

Highly Sensitive Biomarker Detection via Stimuli-responsive Reagents

James Lai[1], Selvi Srinivasan[1], Ian Andrews[1], John Hill[1], Barry Lutz[1], Patrick Stayton[1]

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Clinical diagnostic tests such as immunoassay have been utilized for patient diagnosis to significantly improve health care and reduce costs by detecting trace amounts of certain proteins in patients for identifying harmful cells and troublesome cellular processes. The mainstream immunoassays utilize antibodies immobilized on solid supports for biomarker recognition and separation, which result in long assay time and compromise assay detection limit. In order to achieve higher assay sensitivity, our group has developed stimuli-responsive affinity reagents to address some of the biomarker separation challenges. The reagents such as antibodies conjugated with stimuli-responsive polymers respond sharply and reversibly to physical or chemical stimuli by changing their conformation and physical-chemical properties, i.e. changing from a hydrophilic state to a more hydrophobic state. Stimuli-responsive reagents can replace the antibodies immobilized at solid supports to overcome the mass transport limitations associated with heterogeneous immunoassays because the biomarker binding occurs in a homogeneous solution where molecular diffusion of the reagents facilitates rapid mass transport equilibration. The conjugates can interface with different diagnostic devices to enable rapid immunoassay by facilitating simple and effective biomarkers (or full sandwich immunocomplexes) separation and detection. Additionally, the rapid assay system is scalable to larger starting volumes, which provides opportunities to concentrate dilute biomarkers, thus improving detection ranges and expanding diagnostic options in immunoassays. In this presentation, I will discuss the utilities of stimuli-responsive affinity reagents for microfluidic immunoassay to enable sensitive detection for prostate specific antigen in human plasma as well as rapid biomarker purification and enrichment in human plasma for infectious disease such as HIV.

Session No.: S44-04

Advanced Surface Modifications of Biomaterials for Tissue Engineering and Regenerative Medicine Applications

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Engineering of the surface is the multidisciplinary research of materials science and has a broad range of applications to chemistry, biology, engineering and medicine. Surface modification of a biomaterial can be done by different methods for altering into required characteristics, such as size, morphology, topology, wettability, roughness, surface charge, reactivity, biocompatibility and applicability. Tissue engineering is the use of a combination of cells, engineering and materials methods, and suitable biochemical and physicochemical factors to improve or replace biological functions of tissues. Here we provide an overview of the various applications of our surface modification systems and also discuss successes to date, current limitations and future directions. We developed surface modification system of nanotubes, nanoparticles, polymers, plastic wares, polydimethylsiloxane chips, using chemicals, zwitterionic polymers, polyethylene glycol (PEG), 3-aminopropyltriethoxysilane (APTES), extracellular matrix (ECM), other bioactive molecules, etc for efficiently enhancing cell functions. Our results highlight an innovative surface-modified biomaterials for advance cell culture and tissue regeneration system and may allow the development of enabling technologies for tissue engineering and regenerative medicine applications.
Keywords: surface modification, 2D culture, 3D culture, biomedical engineering, tissue regeneration

Session No.: S44-05

Glucose Delivery System Based-hydrogel Composite Scaffold for Enhancing MSC Survival

Hervé Petite[1], Julie Boisselier[2], Mickael Deschepper[3], Elodie Lefebvre[2], Veronique Larreta Garde[2], Emmanuel Pauthe[2], Mathilde Barbosa[3], Joseph Paquet[3], Joseph Paquet[3]

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Constructs developed for tissue engineering present limited potential due to massive death of transplanted cells after engraftment. This issue can be overcome by in situ supplying glucose that acts as the main metabolic fuel for Mesenchymal Stem Cells in severe hypoxia. In this study, a composite construct is engineered to provide glucose to MSCs and to enhance their survival when transplanted. Two combined strategies are developed. The glucose leakage outside the tissue construct is delayed by increasing the intrinsic viscosity of the fibrin hydrogels by supplementation with starch. The starch polysaccharide serves as the main source of glucose. The long-term supply of glucose is achieved adding a-amyloglucosidase (AMG)- containing poly(lactic-co- glycolic acid) nanoparticles. Sustained delivery of a-amyloglucosidase into the starch-loaded fibrin gel provides continuous production of glucose in situ via the enzymatic hydrolysis of starch. Hydrogels containing fibrin, starch and AMG are self-supported and present a storage moduli superior to 1 kPa. Enzymes containing-nanoparticles are able to produce glucose over a month and allow sustained glucose delivery inside hydrogels. Experiments show that MSCs loaded in these hydrogels and placed either in vitro under hypoxia or ectopically implanted in nude mice have a survival rate significantly higher than MSCs in fibrin gels over 14 days. This work is a proof of concept that an innovativeengineered construct, based on a starch hydrogel containing aamyloglucosidase is able to deliver glucose versus time. In the context of regenerative medicine, it represents a highly pertinent system to enhance Human Mesenchymal Stem Cells survival in cell constructs.

Session No.: S44-06

A Bifunctional Biomaterial with Photothermal Effect for Tumor Therapy and Bone Regeneration

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Bone is the most favored organ for metastatic cancer due to their special microenvironment. However, surgery intervention is difficult to eliminate bone-tumor cells completely. To kill the residual tumor cells, traditional chemo/radiotherapy has been widely used, but the chemo/radiation-resistance and severe side-effect result in endless suffering to patients. Meanwhile, surgery intervention usually leads to large bone defects, which is difficult to be healed by themselves. Photothermal therapy as an effective, non-invasive and low-toxic strategy via hyperthermia has attracted significant attentions. In our study, via 3D-printing and surface-modification strategies, we have designed a new bifunctional biomaterial with excellent photothermal performance for killing the residual bone-tumor cells, and simultaneously to harness the bioactivity of biomaterials for enhancing the healing of the large bone defect after surgical resection of bone tumor. The prepared graphene oxide-modified β-tricalcium phosphate (GO-TCP) composite scaffolds exhibited excellent photothermal effects under the irradiation of 808nm NIR (0.36W/cm2), while no photothermal effects were observed for pure β-TCP scaffolds. The photothermal temperature of GO-TCP scaffolds could be effectively modulated in the range of 40~90℃ by controlling GO concentrations, surface-modification times and power densities of NIR. The distinct photothermal effect of GO-TCP scaffolds induced more than 90% of cell death for osteosarcoma cells (MG-63) in vitro, and further effectively inhibited tumor growth in mice. Meanwhile, the prepared GO-TCP scaffolds possessed the improved capability to stimulate the osteogenic differentiation of rabbit bone mesenchymal stem cells by upregulating bone-related gene expression, and significantly promoted new bone formation in the bone defects of rabbits as compared to pure β-TCP scaffolds. Our results suggest that the prepared GO-TCP scaffolds could be used for therapy and regeneration of large tumor-related bone defects. This work may pave the way for tissue engineering by developing novel bifunctional scaffolds with therapy and regeneration capabilities to solve tumor-related tissue defects.

Session No.: S45-01 Keynote Speaker

Recent Trends and Progress of Silk Based Engineered Biomaterials

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Biomaterials are the key of tissue engineering and regenerative medicine, serve as substrate for tissue reconstruction and facilitate the cross-talk between cells and bioactive molecules by providing suitable platform. Silk biopolymer comprises of two proteinaceous components namely fibroin and sericin; both of which are established as biomedical materials due to cyto-compatibility, bio-degradability mechanical strength and minimal immunogenicity. Moreover, sericin attributes unique features like anti-oxidant, anti-apoptotic and anti-bacterial properties, which are very attractive for wound healing and other applications. All water based processing of silk proteins imparts the flexibility to engineer silk biomaterials into nano to macro-scale for target specific application. Silk based designing features include 2D thin films, 3D porous scaffold, hydrogels, nanoparticles, microcapsules, electrospun nanofibrous mats, and micro/nano composite architectures involving in restoration of the damaged structure and functionality of the engineered tissue (bone, cartilage, skin, cardiac, vascular, alveolar, auricular, lung, corneal and neuron) or delivery of bioactive molecules like antibiotics, growth factors, therapeutic molecules and gene. We will discuss the successful journey of silk from century old commodity textile materials to biomedical engineered materials for tissue regeneration and therapeutic delivery vehicles (Supported by Department of Biotechnology and Indian Council of Medical Research, Govt. of India, New Delhi, India).

Session No.: S45-02

Localized and Controlled Delivery of Curcumin from Injectable Gelatin/Thai Silk Fibroin Microspheres for the Treatment of Osteoarthritis in a Rat Model

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Curcumin, a polyphenol hydrophobic molecule derived from turmeric, exhibits dominant anti-oxidant and anti-inflammation activities. The therapeutic potential of curcumin to treat inflammation-related diseases such as osteoarthritis has been reported. However, bioavailability of curcumin when taken orally is very low due to its water insolubility and short half-life. Therefore, the development of curcumin delivery system is necessary. In our recent work, the gelatin/Thai silk fibroin (G/SF) microspheres at various weight blending ratios were successfully fabricated using water-in-oil emulsion and glutaraldehyde crosslinking techniques. We previously showed that the G/SF microspheres (diameter 194–217 μm) could encapsulate and release curcumin in a sustained manner over 14 days in vitro. In this study, we aimed to apply the previously developed curcumin-encapsulated G/SF microspheres (100/0 and 30/70) for a localized treatment of osteoarthritis in a rat model. The curcumin-encapsulated microspheres were intra-articular injected in monosodium iodoacetate-induced osteoarthritis in Wistar rats. After 1, 4 and 8 weeks of a single treatment, the level of interleukin-6 (IL-6) in blood serum was analyzed by ELISA. At 1 week after treatment, the levels of IL-6 in the serum of rats treated with curcumin-encapsulated G/SF microspheres were reduced, compared to that of non-treated rats. The x-ray and histological examination was performed on articular joint of rats at 8 weeks after treatment. The histologic scores, examined on articular joint (in terms of structure, cell, tidemark, and pannus formation) and synovial tissue change in both synovial lining layer and subsynovial tissue, of the rats treated with curcumin-encapsulated G/SF microspheres was comparable to that of the normal rats. The localized and controlled delivery of curcumin from G/SF microspheres was suggested to be applied for anti-inflammation treatment of osteoarthritis.

Session No.: S45-03

Fabrication and Characterization of Hydrocolloid Dressing with Silk Fibroin Nanoparticle for Wound Healing

Md. Tipu Sultan[1], Ok Joo Lee[1], Bo Mi Moon[1], Hyung Woo Ju[1], Jung Min Lee[1], Hyun Jung Park[1], Ye Ri Park[1], Ju Yeon Jeong[1], Vijay Kumar[1], Yeung Kyu Yeon[1], Chan Hum Park[1]

[1]Nano-Bio Regenerative Medical Institute

Hydrocolloid dressings have been developed for many types of wound healing. In particular, dressing is a critical component in the successful recover of burn injuries, which causes a great number of people to not only suffer from physical but also psychological and economic anguish each year. Additionally, silk fibroin is the safest material for tissue engineering due to biocompatibility. In this study, we fabricated hydrocolloid dressings incorporating silk fibroin nanoparticles to enhance the efficacy of hydrocolloid dressing and then use this silk fibroin nanoparticle hydrocolloid dressing (SFNHD) in animal models to treat burn wounds. The structures and properties of SFNHD were characterized using tensile strength and Cell Counting Kit-8 assay. The results indicated the structural stability and the cellular biocompatibility of the hydrocolloid dressing suggesting that SFNHD can be applied to the treatment of wounds. To demonstrate the capacity of a silk fibroin hydrocolloid dressing to treat burn wounds, we compared SFNHD to gauze and Neoderm®, a commercially available dressing. This study clearly demonstrated accelerated wound healing with greater wound structural integrity and minimal wound size after treatment with SFNHD. These observations indicate that SFNHD may be an improvement upon current standard dressings such as Gauze and Neoderm® for burn wounds.

Session No.: S45-05

Development of Scaffold Using the Composite of Silk Fibroin and Polyvinyl Alcohol for Cartilage Regeneration

Vijay Kumar[1], Jung Min Lee[1], Ok Joo Lee[1], Hyung Woo Ju[1], Yeung Kyu Yeon[1], Bo Mi Moon[1], Hyun Jung Park[1], Sultan Md. Tipu[1], Ju Yeon Jeong[1], Ye Ri Park[1], Chan Hum Park[1]

[1]Nano-Bio Regenerative Medical Institute

The lack of regeneration and the self-healing ability of cartilage tissues consists the great challenge to address cartilage disorders such likes congenital malformation and trauma in human. Silk fibroin is a successful biomaterial in cartilage tissue engineering due to its excellent biocompatibility and bioabsorbable properties. However in order to enhance the mechanical strength, polyvinyl alcohol (PVA) is a best additional polymer to prepare a hydrogel. In this study, we investigated Silk/PVA porous ear-shaped hydrogel scaffold for auricular cartilage regeneration. To control the shape of human's ear structure, we accurately designed the ear model by 3D printer and a Polylactic acid (PLA) ear mold was created. The silk/PVA ear-shaped hydrogel scaffold was fabricated by using salt leaching method and was incubated in freeze-thaw cycles for three days. The morphological, mechanical properties were characterized, and cartilage regeneration study was carried out in vitro and in vivo conditions. The histological study was done with Masson's trichrome and Hematoxylin & Eosin staining that confirmed the lucunar structure and neo-cartilage formation appeared in two weeks specimens. The cartilage maturity was increased in four weeks where in six weeks shows the efficient matured cartilage with typical lucunar structure. The similar results were confirmed in vitro study. We achieved engineered auricular cartilage in the desired 3D ear-shaped using silk/PVA hydrogel. This study could be useful for auricular cartilage regeneration and engineering.

Session No.: S46-01 Keynote Speaker

Therapies of Bone Regeneration to Promote Osseointegration of Dental Implants

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Dental implants are considered as the treatment of choice for replacing missing teeth in elderly people. The clinical performance of dental implants has been attributed to their firm osseointegration. However, much research has addressed implant treatment aspects in challenged osteoporotic conditions. Implant surface modification is considered as an important approach to favor the process of osseointegration. For instance, implant surface topography at the micro/nanoscale level offers a significant role in anchoring cells and connecting to surrounding tissues, thereby promoting peri-implant osteogenesis. In addition, the deposition of bioactive (instructive) coatings onto the implant surface has been explored. Inorganic (CaP)-based implant coatings show the ability to directly bond to bone tissue and increase the biochemical interlocking between bone and surface materials. Other efforts focused on the deposition of biological molecules, such as extracellular matrix (ECM) proteins collagen, enzymes, and growth factors. ECM-proteins are found to enhance implant osseointegration through the accelerated speed and amount of new bone formed at the interface. More recently, new therapies strategies include the development of surface drug-loading system to locally target bone disorders around dental implants more effectively. Anti-osteoporotic agents (e.g. bisphosphonates and statins) found to promote bone healing related to titanium implants in challenged bone conditions (e.g. osteoporotic bone). When these medications are released gradually and locally in the peri-implant area, the bone-to-implant healing process might be increased. From the aforementioned, it appears that there are many therapies of bone regeneration to promote the biological performance of dental implants. However, the exact underlying biological mechanisms of these therapies have not been fully characterized. Consequently, our research is focusing on the in vivo investigation of new therapies of bone regeneration to achieve the desired biological responses around implants, especially in compromised conditions.

Session No.: S46-02 Invited Speaker

Bioceramics-based Tissue Engineering Research and Its Clinical Application in Indonesia

Ika Ana[1], Anne Handrini Dewi[1], Retno Ardhani[1]

[1]Universitas Gadjah Mada

The bulk of the Indonesian medical device market is supplied by imports. Thus, to contribute to the development of the country, our research group has been being successful to valorize the technology in developing and fabricating CHA (carbonate apatite) composite from indigenous bioresources as bone substitute (for the purpose of bone tissue engineering). The overview and data on the current status of bioceramics based tissue engineering research and its application in Indonesia are provided in the article. Furthermore, it is also presented here the use of Calcium sulfate, which has paid attention in regenerative dentistry. Calcium sulfate (CS) or also known as POP (Plaster of Paris) is a self-setting, biocompatible and osteoconductive material with a long history for the treatment of skeletal defects. However, calcium sulfate cements shows a too fast resorption rate and is unable to provide a long term 3D framework during the osteogenesis process. This has been challenges in the area of bioceramics. In view of this, our research group has successfully introduced the controlled release technology into the POP system to overcome the problem and enhanced properties of POP. Nowadays, the use of bioceramics- based tissue engineering for bone substitution purposes, haemostatic sponge, scaffold for stem cells and drug delivery technology of metronidazole for periodontal diseases resulted by our group have been being translated into either clinical use or commercialization.

Session No.: S46-03 Invited Speaker

Curcumin Release Based on Chitosan Nanofibers for Wound Healing Applications

Thien Doan Van Hong[1]

[1]Can Tho University

Chitosan (CS) nanofibers were fabricated by an electrospinning method. The characteristics and surface morphologies of the CS nanofibers were observed under a Scanning Electron Microscope (SEM). The diameters of CS nanofibers were in the range of 100 to 250 nm. The chitosan nanofibers were applied for curcumin loading and delivery. The encapsulation efficiency was 95% and loading capacity was up to 42% (g curcumin/ g chitosan nanofibers). Then, the release profiles of curcumin were investigated. The release profiles could be divided into two stages: the rapid release within the first 2 hours with about 50% curcumin release; the sustained release thereafter. Thus, CS nanofibers would be potential carriers of curcumin for wound healing applications.

Session No.: S46-04 Invited Speaker

Photocrosslinked Hydrogels and Electrospun Scaffolds in Cardiovascular Tissue Engineering

Anwarul Hasan[1]

[1]Qatar University, American University of Beirut, and Harvard Medical School

Cardiovascular diseases are among the leading causes of death worldwide. Tissue engineering and regenerative medicine have emerged as potential solutions for many cardiovascular diseases such as development of implantable tissue engineered vascular grafts for bypass heart surgeries and regeneration of cardiac tissue after tissue-damage or tissue-death resulting from cardiac arrests. A major obstacle for the widespread use of tissue engineering in clinical applications is the lack of suitable biomaterials with required combination of biomechanical and biological properties. Photocrosslinkable mcro-porous hydrogels and electrospun nano-microfiber scaffolds have been widely investigated for application in tissue engineering and regenerative medicine. This talk will present some interesting results from our recent studies on potential applications of Photocrosslinkable methacrylated gelatin hydrogel and electrospun-airjet-sprayed composite scaffolds in cardiovascular tissue engineering and regenerative medicine.

Session No.: S46-05 Invited Speaker

Molecular Therapy for Bone Regeneration

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Bone healing is a long process that takes weeks to complete. It becomes even more challenging when patients suffer from bone structure loss like in major accidents or medical conditions like osteoporosis. With the current work aiming towards developing new drugs that aid bone regeneration, we take a closer look at characterizing the types of drugs used. Small molecules are molecules that are commonly used in pre-clinical studies to investigate their ability to regenerate lost bone structure. On the other hand, there are larger protein based molecules that are used. Because they are protein based, the risks of developing negative immune responses are higher. In addition to that, the cost of synthesizing protein based drugs is much higher as well. Small molecules, in most cases, have a lower risk to develop negative immune responses and are cheaper to synthesize. Here we take a deep look at some of the small molecules that have been used in pre-clinical trials and have produced positive bone regeneration results and then separate them into biological and synthetic molecules. Biological molecules are small molecules originating from organic sources while synthetic molecules are the small molecules that are chemically synthesized with no organic origins. We also look at their mechanism of action as well to see if there are major differences between the biological and synthetic molecules in terms of their mode of action.

Session No.: S46-09 Invited Speaker

Adipose Stem Cells for Knee Osteoarthritis: From Preclinical to Clinical Trials

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Bone healing is a long process that takes weeks to complete. It becomes even more challenging when patients suffer from bone structure loss like in major accidents or medical conditions like osteoporosis. With the current work aiming towards developing new drugs that aid bone regeneration, we take a closer look at characterizing the types of drugs used. Small molecules are molecules that are commonly used in pre-clinical studies to investigate their ability to regenerate lost bone structure. On the other hand, there are larger protein based molecules that are used. Because they are protein based, the risks of developing negative immune responses are higher. In addition to that, the cost of synthesizing protein based drugs is much higher as well. Small molecules, in most cases, have a lower risk to develop negative immune responses and are cheaper to synthesize. Here we take a deep look at some of the small molecules that have been used in pre-clinical trials and have produced positive bone regeneration results and then separate them into biological and synthetic molecules. Biological molecules are small molecules originating from organic sources while synthetic molecules are the small molecules that are chemically synthesized with no organic origins. We also look at their mechanism of action as well to see if there are major differences between the biological and synthetic molecules in terms of their mode of action.

Session No.: S46-06

Preparation and Characterization of Acellular Porcine Pericardium for Tissue Engineering Application

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The aim of this study was to fabricate and characterize acellular porcine pericardium that can be used as a patch for cardiovascular repair. Porcine pericardial tissues were treated in 10 mM Tris-HCl, sodium dodecyl sulfate (SDS) at different concentrations and time points. Optimal decellularization protocol was determined according to clearage of cellular components and DNA via histology and DNA quantification, respectively. There were no significant changes in stress and fracture strain after decellularization. Liquid extracts from decellularized pericardium (dP) caused no cytotoxicity towards human fibroblasts. dP was capable of supporting an appropriate attachment and proliferation of human endothelial progenitor cells (hEPCs) and human adipose derived stem cells (hADSCs). Moreover, dP did not cause local inflammatory effect after 30 days of transplantation in mice.

Session No.: S46-07

Dual Delivery of Platelet-derived Growth Factor and Vascular Endothelial Growth Factor by Silk/Calcium Phosphate/ PLGA Based Nanocomposite Scaffold

Mehdi Farokhi[1], Fatemeh Mottaghitalab[2], Mohammad Ali Shokrgozar[1]

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To exploit the therapeutic potential of growth factors in tissue regeneration, it is necessary to design a porous scaffold in order to concurrently accommodate cells and release angiogenic factors in a controlled manner. In an attempt to address these issues, we developed a nanocomposite scaffold based on silk/calcium phosphate/PLGA by freeze-drying and electrospinning methods in order to control the release of platelet-derived growth factor (PDGF) and vascular endothelial growth factor (VEGF). The highly porous scaffold possessed appropriate chemical and physical structure as confirmed by FTIR, XRD, SEM, and dynamic light scattering (DLS). Furthermore, the incorporation of PDGF and VEGF in the scaffold was confirmed using Raman spectroscopy while their bioactivity was maintained by 82% and 89% for up to 28 days, respectively. The release of PDGF was slower than VEGF as respected. Additionally, the scaffold could promote proliferation, alkaline phosphatase production and attachment of human osteoblast cells. Histological examination established new bone matrix formation with neovascularization in the angiogenic factors loaded scaffold after 10 weeks of implantation in rabbit model. Finally, it was considered that the fabricated nanocomposite could be useful for bone tissue engineering applications.
Key words: Silk, PLGA, VEGF, PDGF, Bone tissue engineering

Session No.: S46-08

Method for Evaluating Actin Cytoskeleton Patterns for Tissue Engineering Research

Jeremy Teo[1], Yanthe Pearson[1], Matthew Martin[1], Nicolas Christoforou[1]

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The significant gap between quantitative and qualitative understanding of cytoskeletal function is a pressing problem; microscopy and labeling techniques have improved qualitative investigations of localized cytoskeleton behavior, whereas quantitative analyses of whole cell cytoskeleton networks remain challenging. Here we present a method that accurately quantifies cytoskeleton dynamics. Our approach digitally subdivides cytoskeleton images using interrogation windows, within which box-counting is used to infer a fractal dimension (Df) to characterize spatial arrangement, and gray value intensity (GVI) to determine actin density. A partitioning algorithm further obtains cytoskeleton characteristics from the perinuclear, cytosolic, and periphery cellular regions. We validated our measurement approach on Cytochalasin-treated cells using transgenically modified dermal fibroblast cells expressing fluorescent actin cytoskeletons. This method differentiates between normal and chemically disrupted actin networks, and quantifies rates of cytoskeletal degradation. Furthermore, GVI distributions were found to be inversely proportional to Df, having several biophysical implications for cytoskeleton formation/degradation. We additionally demonstrated detection sensitivity of differences in Df and GVI for cells seeded on substrates with varying degrees of stiffness, and coated with different attachment proteins. This general approach can be further implemented to gain insights on dynamic growth, disruption, and structure of the cytoskeleton (and other complex biological morphology) due to biological, chemical, or physical stimuli. In the field of tissue engineering and regenerative medicine, phallodin stains have become a standard assay for qualitative assessment of cyto-compatibility, morphogenesis, and tissue development. Our technology reduces the subjective descriptives of the results obtained from such assay and enhances research functionality through the increased information extraction and standardization of measurement.

Session No.: Y01-01

New Design of Ionic Copolymers for Biomedical Coatings

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A material with positive charges may increase its adsorption to blood cells, while that with negative or zwitterionic charges may display anti-fouling effect on proteins and blood cells. Therefore, plenty of research is focusing on the preparation of polycations, polyanions, and zwitterionc polymers. However, the hydrophilic surface may create weak interaction between cells and surfaces. In order to investigate the interaction of polymer coatings with blood, we employed a series of charged copolymers, being easily spin-coated on device surface to explore their non-specific absorption and selectivity toward proteins, blood cells, and fibroblast cells. In order to inhibit the polymer dissolving to aqueous mediums when the coatings contact with blood, cells, or body fluids, a hydrophobic portion in a polymer composition should be carefully considered. Some acrylic monomers with various long hydrocarbon chains are often used to increase the hydrophobicity of copolymers. However, consideration of moderate contents of ionic monomers in the compositions always limits the use of copolymers. We introduced a new monomer with a soft ether linkage and a polymerizable acrylate group, showing excellent biocompatibility. A series of polymerization process were carried out for the exhibition of copolymeric properties. The hydrophobic ether-type monomers performs flexible property and sticky to a coated substrate, which collaborates with hydrophilic ionic portions to show specific biomedical functions. We identified the chemical structures of copolymers with NMR, GPC, and FTIR. Then the copolymers were coated on substrates by spin-coating and characterized for surface properties by FTIR-ATR and a contact angle analyzer. L929 fibroblast cells were used in MTT assay to confirm the cytotoxicity of various ionic coatings and their elutions. Cell adhesion on coated surfaces were performed to evaluate the stimulation of cell growth. In blood-cell adhesion measurements, some compositions of copolymers displayed anti-fouling property or preferentially attachment for some blood cells.

Session No.: Y01-02

Coacervated Mussel Protein as an Adhesive Delivery Carrier for Stem Cells

Tae Yoon Park[1], Hyo Jeong Kim[1], Bong-Hyuk Choi[1], Hyung Joon Cha[1]

[1]Postech

Stem cell therapies are emerging branch of medicine for patient with chronic disease as the alternative of organ transplantation. Especially, in the case of myocardial infarction (MI), as the limited self-regeneration capacity of the adult heart, cell-based therapies are potential for treating and preventing cardiac dysfunction. However, due to blood circulation with high pressure of heart, only less than 10% of injected cells without carrier can be retained. Thus, future therapeutic methods for MI aim the increasing cell retention after injection, which is directly related to efficacy improvement. This study developed adhesive coacervate cell delivery system, which is composed of mussel adhesive protein (MAP) and hyaluronic acid (HA), to efficiently retain cells in infarcted area after cell transplantation. As one of ECM molecules, HA helps to interact with stem cells via cell surface receptors. As the interfacial tension of coacervate is very low, it can easily encapsulate stem cells. Adipose-derived stem cells could be successfully encapsulated (~99%) in the MAP-HA coacervate and be alive over a week with their stemness. In addition, as the MAP-HA coacervate is water-immiscible with strong adhesion due to 3,4-dihydroxyphenylalanine (DOPA) residues, it was stable under the wet condition with high shear stress. Thus, MAP-HA coacervate might have a potential to deliver cells into infarcted area as cell delivery carrier for stem cell therapy. Furthermore, we expect that the coacervate can retain growth factors secreted by cells and control their releasing.

Session No.: Y01-03

Two-photon Excited Immobilization of Bmp-2 on Micrometer-scaled Protein Structure

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Objectives: Technologies on the spatial-temporal control of soluble growth factors such as bone morphogenetic protein 2 (BMP-2) are essential for stem cell niche studies. Multi-photon-based biofabrication is an emerging technology able to improve the spatial resolution of conventional fabrication techniques by precisely fabricating protein structures utilizing femto-second lasers. In the present study, we aim to spatially control the presentation of BMP-2 by fabricating pre-designed BMP-2 patterns on protein structures via two-photon excited immobilization technique. Methods: The protein matrixes with dimension of 101μm × 101μm × 5μm were firstly fabricated by photo-crosslinking Bovine Serum Albumin (BSA) (300 mg/ml) in the presence of photosensitizer, Rose Bengal (RB) (0.1%, w/v), which was activated by femto-second laser with output power of 188 mW at wavelength of 800 nm. Then the protein matrixes were rinsed with phosphate buffer saline (PBS) followed by reloading with a mixture of recombinant human BMP-2 (rhBMP-2) (5 μg/ml) and RB (0.1%, w/v). The photo-crosslinking of BMP-2 patterns with dimension of 20 μm × 20 μm × 2 μm were exerted on the BSA matrixes with different laser powers and scan cycles. Afterwards, the efficacy of BMP-2 photo-crosslinking was verified by immunofluorescence staining. Results: BMP-2 can be immobilized by two-photon laser in the presence of Rose Bengal in a dose-dependent manner of laser power and scan cycle. The range of laser power required for fabrication of visible BMP-2 patterns is from 20.6 mW to 62.0 mW and the according scan cycle is 1 and 5, respectively. Conclusions: Our current study provides a novel platform for spatial control the presentation of growth factor such as BMP-2. Investigation on the bioactivity of cross-linked BMP-2 is underway.

Session No.: Y01-04

Novel Fabrication Method of Peritoneal Dialysis Filter Using Silk Fibroin with Urease Fixation System

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During the last decade, there has been a great advanced in the kidney dialysis system by wearable artificial kidney (WAK) system for end-stage renal disease patients. Uremic solute removal and water regeneration system are the most prerequisits for WAK to work properly. In this study, we designed a filtering membrane system by using immobilized urease silk fibroin filter and ecaluated its comparative effectiveness with a PVDF filtering system in peritoneal dialysate regeneration and urea removal efficacy. We compared this memebranes's characteristics and performances by SEM-EDX analyzerand ability of removal usea, water-binding abilities and porosity test, urea removal rate, cytotoxicity assay,enzyme activity assay.Under the condition for optimization of urease, the percentage removal of urea was about 40% and 60% in urea immobilized PVDF and silk fibroin scaffolds, respectively. The batch experimental result showed that immobilized filter removed more than 50% of urea in 50mg/dL urea solution. We suggest that silk fibroin with urease fixation filter can be used more effectively for peritoneal dialysis solute regeneration system, which have hydrophilic property and prolonged enzyme activity.

Session No.: Y01-05

Fabrication and Evaluation of Pancreatic Islet-like Tissues Containing ECM Microparticles

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Reconstruction of functional pancreatic islet-like tissues in vitro is one of the important issues for developing efficient therapeutic approaches for type 1 diabetes mellitus. Various studies have been reported to fabricate islets-like tissues from pancreatic islet cells, including the formation of multicellular spheroids and stacking of cell sheets. However, it is usually difficult to reconstitute ECM components with micro-scale precision in such closely packed tissues. We have previously developed processes to prepare microparticles made of ECM proteins, and applied the particles to the fabrication of composite multicellular spheroids comprising ECM particles and cells. In this study, we proposed a technique to fabricate islet-like tissues by forming islet tissue-like spheroids using ECM microparticles and evaluated their functions.. In the experiment, we first prepared ECM microparticles made of type I collagen or Matrigel using membrane emulsification techniques. An aqueous solution of ECM components was extruded into a water-soluble organic solvent through a porous membrane to form microdroplets. The droplets were dehydrated and the ECM molecules were concentrated, resulting in formation of the microparticles. Next, the obtained ECM microparticles were mixed with β cells (Min6 cells) and they were seeded to non-cell-adhesive microwell plates to form composite multicellular spheroids. As a result, we successfully obtained microparticles made of collagen and Matrigel with the diameter of ~10 μm. Next, two types of the microparticles were individually mixed with β cells at different ratios (particles:cells = 1:1 or 4:1) and seeded into the microwells. After 2 days of cultivation, islet-like microtissues comprising ECM particles and β cells were successfully formed. Now we are evaluating the functions of the obtained islet-like tissues by evaluating the insulin secretion ability. The proposed 3D culture systems might be potentially advantageous because of the biochemical similarity with microenvironments in vivo.

Session No.: Y01-06

Use of Injectable Light-induced Gold Nanoshells-embedded Silk Fibroin Hydrogel for Combined Photothermal-chemotherapy for Breast Cancer

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Many therapies, including surgery, chemotherapy and radiotherapy, are used to treat cancers, but most of them does not accomplish the desired purposes. For example, it is still a challenge to efficiently kill cancer cells without hurting normal tissues. Alternatively, photothermal therapy is an approach for local ablation of tumor tissues, by using absorbing nanoparticles to convert near-infrared (NIR) light into local heat. Generally, the administrated nanoparticles predominantly accumulate in tumor via the EPR effect. However, the nanoparticle retention time is usually short, since the nanoparticles will be removed quickly via lymphatic system. Therefore, intense laser irradiation for one-shot treatment is necessary to efficiently kill cancer cells. Herein, we developed a novel injectable multifunctional hydrogel system, by embedding gold nanoshells (GN, NIR-light absorber) and doxorubicin (DOX, anti-cancer drug) in a biodegradable natural polymer, silk fibroin. First, the hybrid solution was orthotopically injected into the mice bearing with 4T1 breast cancer and followed by a laser irradiation. The light-induced heat from the GN would increase the local temperature in the tumor and induced the formation of silk hydrogel via sol-gel transition. In this case, the GN and DOX could be trapped well around the tumor and thus the retention time of both agents would be significantly prolonged. Noteworthily, we could perform multiple irradiation treatments at low intensity to induce hyperthermia and trigger the release of DOX. The preliminary results show that the light-induced temperature of tumor in silk/GN group was ca. 5 oC higher than that in free GN group on day 8 after treatment. Besides, Dox could successively be released from silk hydrogel up to 71 % within 12 days because of the acidic environment, degradation of silk hydrogel, and heat-enhanced diffusion. Further material characterization and in vitro/in vivo evaluations are in progress and will be reported in the conference.

Session No.: Y01-07

Mesoporous Silica Nanoparticles for Hepatocyte-targeted Delivery of Lysine: A Novel Method for Hepatocyte Regeneration

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Chun-yen Lee 1, Chia-Wen Wu 2, Richie L. C. Chen 1, Yung-Te Hou 1 1 National Taiwan University Department of Bio-Industrial Mechatronics Engineering, Taipei, Taiwan 2 National Taiwan University Department of Chemical Engineering, Taipei, Taiwan Abstract- Mesoporous silica nanoparticles (MSNs) are solid materials possessing a honeycomblike porous structure and hundreds of empty channels so that can load relatively large amounts of bioactive molecules than other kinds of nanoparticles. Because of those characteristics, it has been developed recently and regarded as an efficient drug carrier in the drug delivery system (DDS). However, all the DDS system are designed to kill the tumor cells, and there are fewer related-reports about the cell-targeted strategy for enhancing the functionality of targeted cells. In this study, we applied MSNs to carry lysine for hepatocyte culture since lysine has been proved to enhance the mitosis of hepatocyte. Moreover, MSNs are encapsulated with chitosan to enhance the biocompatibility. Results have shown that: (1) The albumin concentration in hepatocyte cultures with 1 mg/mL of lysine was increased 16.5% higher than that in control condition (hepatocyte culture without lysine supplement). (2) MSNs showed no cell cytotoxicity to the hepatocytes in low concentration. (3) The loading capacity of lysine on MSNs and chitosan-coated MSNs are 70% and 60%, separately. (4) MSNs particles showed a negative zeta potential of -15mV compared to the chitosan-coated MSNs which showed a highly positive zeta potential of 48mV. The average particle size of chitosan-coated MSNs was found to be relatively larger than MSNs (322nm and 148nm, separately). (5) Chitosan-coated, lysine-embedded MSNs (CLM) enhanced hepatocyte viability in 7 days of cultures. In conclusion, CLM has a high potential in hepatocyte-target study. Its significant impacts are far-reaching at scientific and industrial aspects and will reinforce our existing research strengths in liver tissue engineering.

Session No.: Y01-08

Surface Modification of Xenotransplanted Pancreatic Islet Using Glycyrrhizin-chitosan Bioconjugate for Diabetes Therapy

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A type 1 diabetes mellitus, an autoimmune disease, results from the destruction of the beta cell in the pancreas. Ideally pancreatic islet transplantation is a promising remedy because of its dynamic regulation of blood glucose level of patients. However, transplanted islets are rapidly eliminated due to host's immune responses. Therefore, patient should administer immunosuppressive agents such as tacrolimus to protect transplanted islets, which could induce several side effects to patients. It was reported that a high-mobility-group-box 1 (HMGB1) protein could play a crucial role in transplanted islet rejection. Therefore, it is possible that attenuation of HMGB1 activity can strongly contribute to successful regulation of immune reactions. Here we modified the islet surface with glycyrrhizin-chitosan bioconjugate to modulate the release of HMGB1 protein. We confirmed that the glycyrrhizin-chitosan bioconjugate could be stably immobilized to islet surface without any damage. Then xenogenically implanted the glycyrrhizin-conjugated islets could cure the blood glucose levels of the mice within normal range more two weeks without any immunosuppressants. For control islet implantation, they were rapidly rejected within a week. Currently, we are doing synergistic effect of glycyrrhizin-chitosan bioconjugate when accompanied with low-dose tacrolimus, a well-know immunosuppressant in islet transplantation. Collectively, we demonstrated the feasibility of glycyrrhizin-chitosan bioconjugate for successful outcome of islet transplantation.

Session No.: Y01-09

Sponge-like Electrospun 3D Blend Scaffolds for Tissue Engineering

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Tissue engineering (TE) has played a vital role in revolutionizing and improving the quality of life by restoring, maintaining or enhancing tissue and organ functions. The purpose of the scaffold-based TE is to develop an ECM mimicking three dimensional (3D) scaffolds by providing appropriate physicochemical stimuli to cells, which is vital in cell attachment, proliferation and lineage specification. Some of the techniques investigated in the fabrication of fibrous 3D scaffolds are 3D printing, micro embossing, fiber felts, needle punch and electrospinning, of which electrospinning owing to its cost effectiveness, robustness and scalability has gained more attention in the scientific community. We can fabricate nano to micron diameter fibers with diverse material choices from synthetic polymers to natural proteins through this technique. Despite these benefits, the 2D spinning suffers from the issue of pores blockage which affect cell penetration into the scaffold thus preventing the formation of a fully regenerated tissue. In this study we propose a one-step modified electrospinning process to arrive at a three dimensional scaffold system with interconnected pores that are formed merely by adjusting the polymer solution formulations. This technique eliminates the complexity involved in other fabrication processes like salt leaching or template assisted scaffold fabrication. Unlike conventional electrospinning, which generate film-like pseudo 3D structure, this invention produces sponge like 3D structures with sufficiently large pores. The as-prepared 3D scaffolds exhibits a unique inter twined sub-micron fibrous morphology can be tailored for use in a wide range of tissue engineering applications ranging from bone regeneration to wound healing applications. Future work involves loading biomolecules into these systems and evaluating their release profiles.

Session No.: Y01-10

Electrospun Cellulose Acetate Phthalate Nanofibrous Scaffolds for 3-D Culture of Primary Chondrocytes and Neurons

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Cellulose acetate phthalate (CAP) is a polymer known for being biocompatible, anti-microbicidal and has recently been employed in the form of electrospun fibers for drug delivery. The focus of the present study was the fabrication of nanofibers via electrospinning process and the assessment of biocompatibility of the nanofibres as a novel scaffold for the 3-D cultures. Various electrospinning parameters such as voltage, distance, flow rate, and needle diameter were optimized to generate nanofibers using homogenous polymer-solvents solution. Out of five, only two solvent combinations showed smooth, uniform and nanoscale fibers analyzed by scanning electron microscope (SEM) and SmatSEM. Further, the FTIR analysis of CAP nanofibres revealed hydrolysis of functional ester groups into acid and alcohol that leads to the degradation of the nanofibers at alkaline pH. Thus, the nanofibrous scaffolds were chemically cross-linked for improving their stability in the alkaline conditions. In order to determine the biocompatibility of the nanofibers, MTT assay was performed using L6 cell line that showed the attachment and proliferation of the cells on the nanofibrous scaffolds. The study then focused on the establishment of primary cell culture on the scaffolds. The 3-D culture of chondrocytes and neurons were optimized and maintained for a period of 15-20 days for their functional study. H&E staining was performed in order to visualize and confirm the attachment and proliferation of the cells. This was followed by SEM analysis which conclusively showed that the cross-linked scaffolds using novel solvents combination were biocompatible with primary neurons and chondrocytes.

Session No.: Y02-01

Anticancer Drug Delivery Based on Ph-responsive Sticky Protein Nanoparticles

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A growing number of new treatments have been focused on cancer therapy over the past few decades, because cancer still remains a major leading cause of death in the world despite the advancement of medicine. In general, chemotherapeutics need a balance between inducing cancer cell death and minimizing the adverse side effects toward normal tissue and organs due to their inherent toxicity. Thus, the stimuli-responsive anticancer drug carriers have been developed to maximize therapeutic efficacy and to reduce toxicity. Here, we present a novel strategy for the controlled anticancer drug delivery using mussel adhesive protein (MAP)-based nanoparticle (NP) system. Anticancer drug-loaded adhesive protein NPs were fabricated through a co-electrospraying process of 3,4-dihydroxyphenylalanine (DOPA)-containing recombinant MAP with an anticancer drug, doxorubicin (DOX). The MAP@DOX NPs exhibited pH-induced drug release profiles by changes in the pH-responsive Fe(III)-DOPA coordination stoichiometry and cytotoxic effects including successful induction of apoptosis on cancer cells. In particular, anticancer effect of NPs is closely related to successful cellular uptake and intracellular localization of NPs. To provide vital information for further application, we proposed the possible mechanism on cellular uptake and intracellular fates of the MAP@DOX NPs in cancer cells. Notably, we confirmed that the localization of MAP@DOX to endocytic organelles such as endosome and lysosome is important for pH-responsiveness of MAP@DOX NPs by inducing dissociation of NPs and release of DOX under lower pH. Collectively, the MAP@DOX NPs could be used as a promising pH-responsive smart drug delivery system for cancer therapy applications with further expansion to general drug delivery system.

Session No.: Y02-02

Treatment of High-fat Diet-induced Diabetes Using Oral Absorbable GLP-1 Gene Therapy

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Oral delivery of therapeutic biomolecules has emerged as a promising field in the treatment of disease. In this study, we designed and developed a oral gene delivery system of glucagon-like-peptide-1 (GLP-1), a neuropeptide and an incretin derived from the transcription product of the pro-glucagon gene, to cure high-fat diet-induced diabetes. The expression plasmid encoding GLP-1 gene was initially shielded with bPEI via charge-to-charge interaction and further wrapped with heparin-taurocholate bioconjugate (HTCA-PP) in order to attain ileal apical sodium-dependent bile acid transporter (ASBT) protein mediated active transport in the intestine. When GLP-1/HTCA-PP complex was orally administered (100 g/mouse), the blood glucose levels of high-fat diet-induced diabetes in mice returned to normoglycemic ranges (~150 mg/dL) more than 5 days. In addition, when the GLP-1/HTCA-PP complex was orally administered every 4 days for one month, the blood glucose levels were stably normalized within 150 mg/dL, continuously. During continuous oral administration of GLP-1/HTCA-PP complex, the body weight of all mice was significantly reduced, although high-fact diet was still maintained. However, in the case of untreated (control) mice, their blood glucose levels did not return to normal levels and their body weight was gradually increased. From the immunohistolotical analysis, anti-GLP-1 antibody was observed around islets in the pancreas organ and also in the small intestine organ. On the other hand, GLP-1/HTCA-PP complex demonstrated negligible toxicities even at very high doses (10 mg/kg) in rat. Our oral gene delivery system of GLP-1 gene combined with HTCA-PP non-viral vectors demonstrated a promising therapeutic potential to cure type 2 diabetes mellitus.

Session No.: Y02-03

Integrated Self-assembling Polypeptide Drug Delivery System Possessing Endogenous Stimulus-responsive and Active Targeting for Metastatic Breast Cancer Therapy

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Breast cancer has become the second leading cause of cancer-related mortality in female worldwide. It is greatly curable if diagnosed early. However, more than 90% of breast cancer-related death is due to cancer metastasis to distant organ such as lung, bone, liver and brain at advanced stage. For many medical cases, metastasis has already taken place by the time cancer is detected. Therefore, inhibiting metastasis and targeting to the secondary tumor in distant organs could be a top priority to cure metastatic breast cancer. The structural stability of delivery vehicle and effective release of encapsulated therapeutic drugs are crucial for drug delivery system. In this study, the biodegradable pH-sensitive nanoparticles composed of natural polypeptides and calcium phosphate (CaP) have been developed. The biostable nanoparticles provide three distinct functional domains: the hydrated PEG outer corona for prolonging circulation time, the anionic PGlu shell for CaP mineralization, and the protonation of PHis shell for facilitating anticancer drug release at target site. The active targeting ligand, LyP1, is served to bind to lymphatic endothelial cells in tumor tissue for the reduction rate of breast cancer metastasis. The mean diameter and morphology of the nanoparticles can be responsive to various pH values. Mineralized-Dox-loaded NPs (M-DOX NPs) demonstrated effective release property of anti-cancer drug with sustained controlled release in acidic condition and prevention of drug leakage at physiological pH value. The fast accumulation of our NPs in MDA-MB-231 metastatic breast cancer cells and antitumor efficacy of LyP1-M-DOX NPs compared with other counterparts was obviously noticed. Moreover, the enhanced accumulation of LyP1-M-DOX NPs in activated endothelial cells suggested the potential inhibitory metastasis of MDA-MB-231 cells. Overall, these results suggested that our biocompatible and pH-responsive polypeptide-based nanoparticles can effectively release anti-cancer drug in acidic condition to obtain sustained controlled release as promising carriers for anti-tumor drug delivery.

Session No.: Y02-04

Dependence of Delivery Performance and Antiglaucoma Efficacy of Antioxidant-functionalized Intracameral Pilocarpine Carriers on Grafting Amount of Gallic Acid

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Functionalization of therapeutic carrier biomaterials can potentially provide additional benefits in drug delivery for disease treatment. Given that this modification determines final therapeutic efficacy of drug carriers, here, we investigate systematically the role of grafting amount of antioxidant gallic acid (GA) onto GN in situ gelling copolymers made of biodegradable gelatin and thermo-responsive poly(N-isopropylacrylamide) for intracameral delivery of pilocarpine in antiglaucoma treatment. As expected, increasing redox reaction time increased total antioxidant activities and free radical scavenging abilities of synthesized carrier biomaterials. The hydrophilic nature of antioxidant molecules strongly affected physicochemical properties of carrier materials with varying GA grafting amounts, thereby dictating in vitro release behaviors and mechanisms of pilocarpine. In vitro oxidative stress challenges revealed that biocompatible carriers with high GA content alleviated lens epithelial cell damage and generation of reactive oxygen species. Intraocular pressure and pupil diameter in glaucomatous rabbits showed correlations with GA-mediated release of pilocarpine. Additionally, enhanced pharmacological treatment effects prevented corneal endothelial cell loss during disease progression. Increasing GA content increased total antioxidant level and decreased nitrite level in the aqueous humor, suggesting a much improved antioxidant status in glaucomatous eyes. This work significantly highlights the dependence of physicochemical properties, drug release behaviors, and bioactivities on intrinsic antioxidant capacities of therapeutic carrier biomaterials for antiglaucoma treatment.

Session No.: Y02-05

Synthesis and Fabrication of Gold Nanoparticle Embedded Biodegradable Scaffolds

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Gold nanoparticles, (GNP)s are considered biocompatible materials with multiple applications in medicine. It is thought to be applicable in local heat treatments from near infrared light for the treatment of diseases such as rheumatoid arthritis, it is also an imaging enhancer for radiology and may be used in the diagnosis of cancer. However, localized delivery of GNPs remains a challenging topic, since the nanoparticles may move around upon injection. Poly(glycerol-co-sebacate) (PGS) is a biodegradable polymer that is mechanically compatible with soft tissue and degrades through surface erosion. Therefore, by combining GNPs with PGS, a novel composite material is synthesized with long-term and localized GNP releasing capability. Through a variety of analytical techniques and instruments, such as Inductively Coupled Plasma-Mass Spectrometer (ICP-MS), Laser Ablation-Mass Spectrometer (LA-MS), Alpha Step, X-ray, and computed tomography scan (CT), the distribution of GNPs in PGS-GNPs and the dynamic release of GNPs from PGS-GNPs in vitro enzyme degradation was observed. With the highly tunable degradation rate of PGS, the GNP release rate is also highly tunable, making controlled local heat treatment through near IR light and GNPs a possible future treatment.

Session No.: Y02-06

Fabrication of 3D Graphene Oxide for Electrical and Fluorescent Based Sensing of Vocs

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Detection of volatile organic compounds (VOCs) emitted from cancerous tumor cells in exhaled human breath allows for early diagnosis of various types of cancers. 3D graphene oxide with a large surface area shows potential for creating sensitive but inexpensive VOCs sensors for point of care use. In this study, 3D graphene oxide was synthesized from graphene oxide suspension, hydroquinone and formaldehyde by employing polymerization. The VOC sensitivity was evaluated by measuring changes in electrical conductivity and fluorescence after surface treatment by calixarene. The VOC surface adsorption of the material was evaluated by measuring the electrical current response in flowing N2 gas over a range of concentrations of acetone or 1-butanol at room temperature. It was observed that the device current correlated well with the VOC concentration. The adsorption of acetone resulted in a decreasing current, whereas the adsorption of 1-butanol resulted in higher measured current during sensing. The 3D graphene oxide device was more sensitive than a 2D graphene film based device because of the higher surface area and the high concentration of oxygen-containing functional groups on the surface. To explore the VOC surface adsorption, the surface was treated with organic material, calixarene. Calixarene is a nano-size cup type material, which permits molecule adsorption of similar size to the diameter of cup. It was revealed that calixarene is fluorescence and the fluorescence intensity changes upon adsorption of the VOCs when observed using a fluorescence microscope. Therefore, 3D graphene oxide devices modified with calixarene can detect VOCs with high sensitivity and selectivity by measuring the both electric and fluorescence properties. These results indicated that 3D graphene oxide is a suitable material for VOCs sensing devices. The advantage of this 3D material is its ability to detect VOCs at room temperature.

Session No.: Y02-07

An Anticancer Drug Embolism Mini-factory and High-throughput Cytotoxicity Sensor Platform Based on Tissue Engineering and Micro-fluidic Techniques

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Cancer was still the No.1 cause of death nowadays, almost all the anticancer agents have severe side effects on normal tissues and organs, as a result, recent studies are focus on the development of nucleic acid drug delivery system for reducing the side effect. On the other hand, liver is the most drug metabolism organ in the body, and is also the primary site for biotransformation, however, the uptake of chemicals/drugs sometimes decrease hepatocyte its ability (e.g., hepatocyte albumin synthesis or urea synthesis ability) while the biotransformation was performed during the drug metabolism procedure. In this research, we developed a chemical/drug metabolism mini-factory and high-throughput hepatotoxicity sensor platform based on tissue engineering and micro-fluidic techniques for chemical/drug metabolism. This system was composed of two parts including liver and cancer-on-a-chip platform, separately. Statin, a drug that can lower the use cholesterol, was used as case study. Furthermore, the metabolite of statin after biotransformation was checked its ability for killing the tumor cells on the cancer-on-a-chip platform. Results have shown that: (1) The mitochondria activity on collagen-coated PDMS showed 50% superior than in normal PDMS at 3 days of culture; (2) There are no cytotoxicity to both DU145 cell line and hepatocyte with lower concentration (10-5 mg/mL) statin treatment; (3) The mitochondria activity of DU145 cell line after statin metabolite treatment (statin was biotransformed in liver-on-a-chip platform and therefore formed statin metabolite), was 30% lower than in statin treatment. This system not only reduces the expense, expedites the experiment, but more importantly minimizes the use of living animals and investigates fundamental mechanisms of statin on liver and other organs. The outcomes obtained from this research can be applied as a unique approach to understand and examine the mechanism of anticancer drug. Its significant impacts are far-reaching at scientific and industrial aspects

Session No.: Y02-08

Immediate Naked-eye Detection of Volatile Biogenic Amines from Perishable Foods - A Novel Plasmonic Biosensor Based on Metal Nanoparticles Array Immobilized on Flexible Substrates

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Biogenic amines (BAs) are a family of organic bases that may be found in foods such as fish and meat. The presence of BAs is the result of the decarboxylation of certain amino acids by microorganisms in these foods. The presence of microorganisms in food products not only poses a potential harm to human health, research has also found specific BAs (e.g. histamine) to cause headaches, diarrhea, edema, and other adversities. Thus, the simple, sensitive, and low-cost detection of BAs is of great importance in food safety. Methods such as high-performance-liquid-chromatography (HPLC) and gas-chromatography-mass-spectrometry (GC-MS) have been employed in the sensing of BAs. However, tedious pretreatments, long reaction times, and high equipment costs limit the practicability and applicability of these techniques. Localized surface plasmon resonance (LSPR) of metal nanoparticles (NPs) has been utilized for chemical and biological sensing. In particular, the collective oscillation of electrons within the NPs upon irradiation of incident light is sensitive to changes in environmental refractive index, making NPs suitable for developing plasmonic sensors. Herein, a system of two different types of monolayer nanoparticle arrays immobilized on flexible polyethylene terephthalate (PET) substrates was fabricated for the detection of BAs. For proof of concept, we spiked various concentrations of putrescine in fresh salmon samples purchased from the local market. Visible color change was observed in the silver nanoparticles (AgNPs) biosensor by the naked-eye within 15 seconds, with a limit of detection (LOD) of 43 ppm. The gold nanoparticles (AuNPs) biosensor showed obvious spectral peak shifts, with its LOD as low as 3 ppm. Both biosensors demonstrated LODs well below the European Commission’s recommended concentration of 300 ppm and provided a biosensing platform for the low-cost and simple detection of BAs. Further optical measurements and analyses are in progress and will be presented at the conference.

Session No.: Y02-09

Programmed Transdermal Delivery of Painkillers Using Light-triggerable Microneedles

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In this study, we used biodegradable polycaprolactone (PCL), encapsulated analgesic agent – lidocaine and lanthanum hexaboride (LaB6) nanoparticles (NPs), to make remotely triggerable microneedle (MN). We focused on the feasibility of MN transdermally delivering analgesic agents and its safety assessment of clinical application. Lidocaine content in each MN patch was 1.58 ± 0.17 mg (n = 4). After giving treatment of NIR, LaB6 NPs encapsulated in MN could convert the energy into heat leading to the melt of PCL and release drugs. The skin insertion tests showed that the microneedles could be fully inserted into the skin with penetration depth of 500~600 μm. The amount of released drugs can be controlled by adjusting the irradiation periods and exposure time and MN could be retriggered at least 6 cycles (3 min/cycle). Both skin scald observation and histological section confirmed that microneedles with 6 minutes NIR exposure performed the least heat damage to the skin tissue. The wound could recovered back to the original state after 12 hours. These result shows that there was no obvious heat damage in skin tissue after 6 minutes NIR exposure. We used inductively Coupled Plasma-Mass Spectroscopy (ICP-MS) to prove that LaB6 NPs did not exist in the MN puncher site neither in other specific tissues after 24 hours from MN been applied. These results suggest that remotely controlled microneedles can be used safely under 6 minutes NIR exposure without any obvious damage to the skin tissue and preliminarily reassure its using safety.

Session No.: Y03-01

Creation of Cell-derived Extracellular Matrix of Both Random and Align ECM Scaffold as a Template for Vascular Tissue Engineering

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Biomedical researchers found great interest in extracellular matrix (ECM) scaffolds derived from cultured cells for tissue engineering applications. ECM scaffolds can be prepared from autologous cells to generate autologous ECM (aECM) scaffolds. It can avoids the undesired hostresponses that may be induced by allogenic or xenogenic materials and circumvents the limited supply of autologous tissues. In this study, we first fabricate random and align PLGA meshes as a template for cell culturing using PLGA electrospun fibres. Afterward, Human adipose stem cell (hASCs) were seeded onto the PLGA template with growth medium and endothelial differentiation medium for comparison. Cell-ECM-PLGA constructs were formed by culturing cells in the PLGA mesh for five to six days. Finally, the whole construct was decellularized by freeze-thaw cycling and NH4OH aqueous solution treatment to remove the undesired PLGA template. The ECM left behind will be freeze-dried and sterilized. hASCS will then be seeded onto the ECM scaffold again for further cell-test. The scaffold was also tested in vivo by using Chick Chorioallantoic Membrane (CAM) assay. The results showed that the ECM scaffold provided wonderful effort in angiogenesis. And the ECM scaffold fabricated in the align PLGA mesh showed a better result compare to the random PLGA meshes.

Session No.: Y03-02

Silica Nanoparticle-generating Engineered Mussel Glue for Osteoconductive and Osteoinductive Coatings of Titanium Implants

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Surface chemistry and topography of implants strongly affect osteogenic cell behaviors and bone formation. For dental and orthopedic applications, silica has been concerned as a promising material with osteogenic activity for differentiation and mineralization of osteoblasts. In this work, we describe a novel bioinspired approach to generate silica nanoparticles (SiNPs) using bioengineered musel glue, which is recombinant mussel adhesive protein (MAP) fused with silica-binding peptide. The bioengineered musel glue enabled the efficient coating of titanium surface and the easy generation of SiNPs on the coated-surface by the strong adhesion and silica precipitating abilities, under mild conditions. The bioengineered musel glue plays a role in an organic compartment that protects and maintains stability of nanocomposites as a cushion material between titanium substrate and SiNPs, while inorganic SiNPs stimulate bone cell behaviors as well as build up nanostructures. Moreover, we directly controlled the surface topographies of SiNPs coating with micro-scale features by tuning glue protein coating and silica formation cycle. The osteoblastic cell behaviors such as attachment, focal adhesion, morphology, proliferation, and differentiation were greater on all multilayer SiNPs-coated titanium surface than on bare titanium surfaces. We determined optimal condition of multilayer coating on titanium surface and systamatically investigated how to each topography of multiplaye coatings influences bone cell behaviors. Furthermore, the SiNPs layer was fully generated on various metal surfaces including aluminum, stainless steel, and titanium implant fixture. The mussel-based SiNPs coatings can be successfully employed to enhance osteoconductivity and osteoinductivity in medical implant and bone tissue engineering applications.

Session No.: Y03-03

Fabrication of PLGA/Tricalcium Phosphate Composite Cements for Generating Connective Porous Structure

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Bone prosthetics require biocompatibility and bioabsorbability for regenerating fractured bone tissues. Calcium phosphate cements (CPC) with self-hardening property can fill up bone defects due to its good injectability; however, it's still difficult to control internal structures of the cements. In this study, we fabricated poly(lactic-co-glycolic acid) (PLGA)/tricalcium phosphate composite cements in order to control the mechanical strength and generate connective pores accompanying with decomposition of PLGA. The effects of addition of PLGA particles on the compressive strength and porous structures were investigated. CPCs were prepared by mixing alpha-TCP particles, citric acid (CA), and tilapia scale collagen (Col) or hyaluronic acid (HyA) as a dispersant. The alpha-TCP particles with different particle sizes were employed; first one was spray-dried (SD; 14 um), second freeze-dried (FD; 45 um), and third sintered after molding with cold isostatic press and crushed (CIP; 135 um). The CPC cement with 80% of SD and 20% of FD particles including Col showed the highest compressive strength at 33 MPa for 1 day compared with other cements. PLGA spherical particles at two different particle sizes from 100 to 200 um and from 150 to 400 um were prepared with a double emulsion method using dichloromethane and polyvinyl-alcohol/distilled water. The compressive strength of the CPC cements including 40% of PLGA and Col with the same self-hardening condition at above showed 4.0 MPa which was almost similar to that of human cancellous bone. SEM images and microscopic Raman spectra showed the growth of DCPD at the interface between CPC and PLGA particles, which could be attributed to decomposition of PLGA in the self-hardening process. Later, we plan to coat on PLGA surface to enhance the binding between them.

Session No.: Y03-04

Mutual Influence of Mesenchymal Stem Cells and Meniscal Cells by 3D Co-culturing to Build up a Meniscus Model

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Meniscal injuries result in the deterioration of the meniscal tissue and articular cartilage. The healing potential of the avascular region of the meniscus is limited and no optimal treatment strategy is available. Replacing torn menisci by a tissue-derived 3D meniscus model seems to be a promising strategy. Mesenchymal stem cells (MSC) co-cultured with chondrocytes have been shown to provide a regenerative environment through their chondrogenic differentiation potential, enhanced chondrocyte proliferation and matrix formation. MSC under 3D co-culture conditions influenced by meniscal cell (MC) signaling may promote the modelling of meniscal tissue accordingly. MC were isolated out of an equine meniscus followed by immunohistochemical characterization of fibrocartilage markers. MSC were isolated out of equine bone marrow by gradient centrifugation and characterized by FACS analysis of MSC markers. The adipogenic, chondrogenic and osteogenic differentiation potential were confirmed by histological stainings. Isolation, in vitro expansion and characterization with cell specific markers were successfully achieved for primary MC and MSC. To develop a 3D meniscus model, various static co-culture models of MC and MSC were introduced. MC and MSC were co-cultured on an acellularized porcine-derived jejunum segment (SIS-muc). As second model, MC and MSC were embedded in a Collagen type I hydrogel. As third approach, MC and MSC embedded in the Collagen type I hydrogel were combined with SIS-muc. Different ratios of MC to MSC were investigated by immunohistochemical analysis and quantitative GAG/DNA assays. Static 3D co-culture models showed high viability and resulted in subsequent differentiation of MSC into MC supporting meniscal tissue modeling. MMP2 and MMP9 upregulation confirmed matrix remodeling. The here reported study suggested 3D co-culture of MSC and MC embedded in a Collagen I hydrogel on SIS-muc as suitable biomaterial combination for meniscus tissue engineering. Dynamic co-culture in a vascularized scaffold to build up a functional meniscus implant are under investigation.

Session No.: Y03-05

Synthesis of Biodegradable Polymer with UV-curing

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The development of biodegradable materials with elastomeric properties had become one of the most popular research topics in the past decade, and the need to produce new elastomeric polymers in large scale for a wide variety of applications had been ever increasing. Based on the synthesis of poly(glycerol sebacate)-based photocurable biodegradable polymer, poly(glycerol sebacate) acrylate (PGSA), vinyl acetate (VAc) is added to increase the mechanical properties while maintaining the biodegradability. With UV-curing, the mechanical property can be more stable and most importantly is that the shape of polymer can be controlled by additive manufacturing. The mechanical properties of PGSA are obtained with respect to their Young's modulus from 0.12 to 7.72 MPa, ultimate tensile strength between 0.1 and 1.67 MPa and strain to failure from 121% to 39% by changing the degree of acrylation. With the addition of VAc, the Young's modulus is expected to be enhanced to 10 MPa. The demand for biodegradable medical devices and implantable polymers for clinical use had been increasing along with the continuous growth of population. Biodegradable photocurable polymeric material coupling with additive manufacturing provides a fast and customizable option to make biocompatible, biodegradable and disposable devices.

Session No.: Y03-06

Development of Micropatterned Nanofiber Using Femtosecond Laser to Build Biomimic Structure and Function of Endothelium.

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Endothelium, the inner layer of blood vessel, has elongated structure induced by blood flow, and this structure is associated to special function of barrier related to anti-thrombosis and regulation of immune cell binding. Thus, there have been many attempts to mimic both structure and function of native endothelium as artificial vascular graft. In this study, we fabricated the micropatterned poly(L-lactide) (PLLA) nanofiber using femtosecond laser to mimic the elongated structure of endothelium. Characterization studies showed that laser ablation did not affect the mechanical properties of nanofiber. The surface-patterned PLLA nanofiber guided the migration and elongation of endothelial cells by only topologically aligned groove, not other dynamic flow. Endothelial cells cultured on micropatterned nanofiber have shown lower aspect ratio and uni-directional spreading tendency than non-patterned nanofiber. Moreover, topological groove on monolayer of endothelial cells controlled by micropatterned nanofiber inhibited the monocyte adhesion despite showing normal expression of E-selectin, because topological grooving might disturb the arrangement of receptors related to monocyte binding. Collectively, our results suggest that micropatterning of electrospun nanofibers using femtosecond laser can be used as a platform for giving topographical cues of vascular graft materials.

Session No.: Y03-07

Monitoring Degradation of Polycaprolactone Scaffold in Vivo by Labeling with Gold Nanoclusters

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Biodegradable scaffolds play an important role in tissue engineering. Monitoring the scaffold degradation in vivo by noninvasive approach is still limited, especially for those synthetic polymers which could not be detected by CT or MRI scanning. In the current study, we developed a CT-trackable scaffold by labeling polycaprolactone (PCL) with bovine serum albumin (BSA)-capped gold nanoclusters. In addition, the BSA-capped gold nanoclusters possess fluorescent characteristic which could be detected by fluorescent imaging. The nanoclusters were synthesized, characterized, and their biocompatibility and toxicity were evaluated in cultured cells. The particles were then incorporated into PCL scaffold by 3D printing. After implantation the scaffolds subcutaneously into the nude mice, degradation of the scaffolds were monitored by CT-scanning at 1 month interval, and the samples were harvested for histological analysis. This new approach provided an accurate and noninvasive way for tracking the degradation of synthetic scaffolds in the body.

Session No.: Y03-08

Drug-releasing Silk Micro-particles Fabricated Using Calcium Carbonate Co-precipitation

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Controlled delivery of therapeutics has been an important challenge not only for drug delivery but as well as in achieving vascularization in tissue engineering. Micro-particles have been identified as good vectors in achieving this goal, however conventional fabrication methods are usually harsh and involve toxic chemicals which risk degrading the quality of the loaded therapeutic and the micro-particle material. In this study, silk fibroin, which is a biocompatible and biodegradable natural polymer with notable mechanical strength, is fabricated into micro-particles using calcium carbonate co-precipitation. Silk was co-precipitated with calcium carbonate (CaCO3) into spherical micro-particles. The CaCO3 is completely dissolved thereafter by lowering the pH, leaving the silk micro-particles behind. The process is not only simple, but also allows a gentle purification technique that result to a consistent morphology of the silk micro-particles. In addition, the synthesis is controllable using parameters such as stirring rate, stirring time and salt concentration. The silk micro-particles produced were characterized using scanning electron microscopy (SEM) and were found to be spherical in shape. The size of the particles produced using the parameters chosen were similar to the size of the CaCO3 templates, ranging from 1.6 to 2.2 µm as stirring speed was decreased. Fourier transform infrared spectroscopy (FTIR) was used to confirm the disappearance of CaCO3 after titration with acid, and also provided evidence of the role of Ca2+ coordination chemistry in stabilizing the silk microparticle. Bovine serum albumin was then used as a model drug to study the loading efficiency and the release profile of the silk micro-particles. The biodegradable silk micro-particles can potentially be used for controlled delivery of drugs, and integrated to biodegradable scaffolds to deliver angiogenic factors vital in functional tissue regeneration.

Session No.: Y03-09

The Strategies to Produce hBMSC Microtissues/SF-PCL Based Cardiac Patches with Promoting Cardiomyogenesis of hBMSC

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Since high similarity between in-vitro microtissues of cells and in-vivo tissues, the strategies to fabricate 3D microtissues has been widely investigated for tissue engineering. Moreover, microtissues would enhance the secretion of cytokines and differentiations of BMSC to specific tissues in-vitro compared to those of 2D cell monolayers have been documented. Here, we reported two strategies to modify microenvironments of surfaces to produce hBMSC microtissues/silk fibroin (SF)-poly (ε-caprolactone) (PCL) based cardiac patches with promoting cardiomyogenesis of hBMSC. The strategies are: (a) Biochemical surface modifications: Grafting hyaluronic acid (HA), CD44 receptors, and GRGD, αvβ3 integrin ligands, of human bone marrow-derived mesenchymal stem cell (hBMSC) to the surfaces of SF-PCL, (b) Regulating the mechanical properties of SF-PCL matrix: Varying β-sheet contents (or crystallinity, %) of SF to regulate the mechanical property (Young's modulus) of substrates for investigating the effects of those methods on the formations of 3D hBMSC microtissues, proliferation, and 5-aza inducing cardiomyogenesis of hBMSC in-vitro. For biochemical surface modification method, 3D microtissues formations of differentiated hBMSC on the HA-GRGD/SF-PCL patch were observed by confocal microscopy with promoting cardiomyogenesis which were about 250 μm width and 30 μm height. For regulating the mechanical properties of SF-PCL matrix, 3D microtissues of hBMSC onto SF-PCL patches with 20 and 30% crystallinity of SF were observed while 2D hBMSC monolayers were found on the patch with 44% crystallinity of SF after three days of cultivation. Notably, in-vitro cardiomyogenesis of hBMSC showed that cardiac specific proteins and genes such as CX43 and Gata4 of hBMSC microtissues/SF-PCL patches had significantly higher expressions than those of hBMSC monolayer on SF-PCL ones ($p<0.05$, $n=3$). In conclusion, 3D hBMSC microtissues/SF-PCL cardiac patches were produced by two different strategies with highly promoting cardiomyogenesis of hBMSC although they were produced in different stages.

Session No.: Y03-10

Simultaneous Control of Cellular Gene Activation and Its Peg-based Hydrogel Microenvironment via Conventional Led Light

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In this study, we demonstrate the LED light dependent control of both cells and matrix. In order to develop a simultaneous matrix degradation and gene activation system, human adipose-derived mesenchymal stem cells were engineered to express VEGF by transfection with gene encoding light inducible dimerizing proteins, and these cells were encapsulated in photodegradable hydrogel modified with hydroxylethyl photolinker. It was shown that blue light was able to decrease modulus of synthesized photodegradable hydrogels. Cells with blue light responsive units were able to activate VEGF upon light irradiation. Our initial observation indicated that simultaneous control of hydrogel degradation and gene activation via blue light was effective and influenced cell morphology and phenotype. Further, for the therapeutic application of the developed light responsive system we applied light responsive system in hindlimb ischemia and wound healing model. With simple conventional blue light, we have demonstrated microenvironmental remodeling and controlled gene activation for tissue engineering applications.

Session No.: Y04-01

Preservation of Human Limbal Epithelial Progenitor Cells on Carbodiimide Cross-linked Amniotic Membrane via Integrin-linked Kinase-mediated Wnt Activation

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[Introduction] Given that the Wnt pathway is a major signaling pathway regulating corneal epithelial stem cells, this study aims to investigate the mechanism of preservation of human limbal epithelial (HLE) progenitor cells on carbodiimide cross-linked amniotic membrane (CLDAM) via integrin-linked kinase (ILK)-mediated Wnt activation. [Materials and Methods] HLE cells were cultured on dishes (HLE/dish), AM (HLE/DAM) or CLDAM (HLE/CLDAM). BrdU label retention, CFE assay, immunoconfocal microscopy, real-time quantitative RT-PCR, Western blot, and effect of ILK silencing or over-expression on Wnt activation and HLE cell differentiation were performed to study the influence of matrix ultrastructure on Wnt activity. [Results] Compared with HLE/dish or HLE/DAM cultures, HLE/CLDAM cultures showed greater BrdU retention and colony formation efficiency and expressed higher levels of p63, ABCG2, integrin β1, and ILK. Nuclear β-catenin and TCF-4 levels were higher in HLE/CLDAM cultures compared with HLE cells cultured on collagen IV, laminin, Matrigel, or DAM. Silencing of ILK in HLE/CLDAM cultures led to decreased levels of nuclear β-catenin, TCF-4, and deltaNp63α, whereas cytokeratin 12 expression increased. Over-expression of ILK in HLE/dish cultures had the opposite effects. [Discussion] Carbodiimide cross-linked AM can potentially serve as an artificial corneal epithelial stem cell niche. For the first time, the CLDAM was used as a simulated substrate to investigate this mechanism. We proposed that the CLDAM with its higher rigidity and rougher ultrastructure better preserved HLE progenitor cells in vitro, possibly by activating integrin β1/ILK, which indirectly activated Wnt/β-catenin and subsequently deltaNp63α. [Conclusions] Crosstalk between integrin β1/ILK and Wnt/β-catenin pathway plays a role in HLE cell cultivation.

Session No.: Y04-02

Arginine-Glycine-Aspartate Peptides Functionalized Magnetic Nanoparticles Substrate Modulates Adhesion, Spreading and Differentiation of Human Mesenchymal Stem Cells

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Mechanical stimulation introduced by substrate stiffness has been shown to regulate the differentiation fate of human mesenchymal stem cells (hMSCs). A magnetic twisting device (ferromagnetic microbeads) coated with Arg-Gly-Asp (RGD) sequence that is a well-known ligand for fibronectin receptors of cell surface, such as integrin α5β1, has been used to apply the mechanical stress directly to the integrins under external magnetic fields (EMF) to reveal that integrins act as mechanoreceptors and transmit the external mechanical signals to the nucleus through cytoskeleton to induce different mechanosensitive gene expressions in previous studies. However, substrate stiffness modulated in nanoscale by EMF to regulate hMSCs behaviour has not been explored. In this study, we report a facile platform to investigate the effect of various mechanical stress induced by RGD-integrin interactions on glass substrate under different EMF strengths and orientations on stem cell behaviors. Glass coverslips are silanized for thiol groups using (3-Mercaptopropyl)trimethoxysilane (MPTMS) to conjugate with maleimide groups of PEGylated (MW = 2000) Fe3O4 magnetic nanoparticles (PEG-MNPs). The monolayer of conjugated PEG-MNPs are further conjugated with thiolated RGD peptides. The grafted RGD peptides are semi-mobilized on the glass substrate along the silanized position in response to EMF. Permanent magnets are placed below or above the cell plate for different orientations of the EMF. The application of one or three permanent magnets are responsible for low or high strength of the EMF (namely, LSEMF and HSEMF). Up to now, fluorescence microscopy shows that hMSCs have higher adhesion and spread well with stable actin filamentous structure to the glass substrate when the permanent magnets are placed at the bottom for both LSEMF and HSEMF. In addition, these groups even exhibit enhanced osteogenesis from immunostaining the results of early osteogenic markers. Our platform offers a simple EMF driven platform to modulate hMSCs adhesion, spreading and differentiation.

Session No.: Y04-03

Effect of Macromolecular Crowding in 3D-pellet Culture of Mesenchymal Stem Cell Chondrogenesis

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The extracellular matrix (ECM) component, specifically collagens and glycosaminoglycans (GAGs) plays a vital role in governing the mechanical properties of articular cartilage in generating a functional tissue replacement in cartilage tissue engineering. Hence, the accumulation of cartilage tissue-specific ECM proteins during chondrogenic differentiation of human mesenchymal stem cells (hMSCs) is crucial for regeneration of functional neocartilage. To date, insufficient deposition of specific collagens and GAGs in MSCs generated cartilage represents a bottleneck in cartilage tissue engineering. Macromolecular crowding (MMC) mimicking the physiological crowdedness of in vivo aqueous microenvironment has been shown to enhance thermodynamic activities and biological processes by several orders of magnitude. The impact of MMC on hMSCs chondrogenesis has not been explored. In this study, we investigate the effect of MMC on hMSCs chondrogenic differentiation, under two crowding conditions, continuous crowding or delayed crowding condition, in which crowding was introduced after the initial induction of chondrogenesis. Ficoll 70 and Ficoll 400 were included in standard chondrogenic media to create a crowding condition to 3D-pellet culture. Both continuous and delayed crowding induced significant increased in collagen II and aggrecan mRNA expression, relative to non-crowding condition. Notably, accumulation of collagen II and sGAG in the cartilage pellet tissue increased by 50%, relative to non-crowding condition, evident by both quantitative assays and histological staining. Concomitantly, a significant reduction of soluble collagen II was released into the media of both crowding conditions in comparison to non-crowding condition. Our results suggest that MMC is effective in enhancing cartilaginous ECM tissue deposition. This work demonstrated the potential of MMC application in cartilage tissue engineering, by increasing the biochemical property and hence, the mechanical competency of cartilage generated from MSCs.

Session No.: Y04-04

Light-responsive Gold Nanorod-loaded Tumor-tropic Stem Cells for Cancer Therapy

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The intrinsic tumor-tropism suggests stem cells as a potential cell-based carrier platform for targeted delivery of anticancer agents. Light-mediated therapies such as photothermal therapy (PTT) and photodynamic dynamic therapy (PDT), utilize photon energy to generate thermal energy or reactive oxygen species (ROS), which could effectively trigger cancer cells to undergo apoptosis. To promote the in vivo tumor targeting delivery efficacy of PTT and PDT agents, we proposed to integrate polymer-gold nanorod (AuNR)-based materials with adipose-derived stem cells (ADSCs) for stem cell-based PTT and PDT. AuNRs were surface-modified with thiolated polymers via Au-S linkage to prepare polymer-AuNR conjugates. The Polymer-AuNR conjugates were loaded with photosensitizers, Chlorin e6 (Ce6), with high loading efficiency and stability. Physicochemical properties of the polymer-AuNR conjugates were characterized by UV-Vis spectra, particle size, zeta-potential and TEM imaging. The results indicate that the polymer-AuNR conjugates exhibited good dispersity, high drug loading efficiency, glutathione-sensitive drug release, light-triggered photothermal and photodynamic properties. The in vitro results demonstrate that the polymer-AuNR conjugates were biocompatible and efficiently taken by mammalian cells. Furthermore, upon on light irradiation, the polymer-AuNR conjugates-loaded ADSCs effectively promoted cancer cell death via PTT or PDT bystander effect. Significant tumor growth inhibition and improved survival curve were observed from cancer-bearing mice received intravenous injection of the polymer-AuNR conjugates-loaded ADSCs following by light irradiation.

Session No.: Y04-05

The Immune Property of ESC-MSCs Is Tuned in Inflammatory Microenvironment

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Embryonic stem cell derived mesenchymal stem cells(ESC-MSCs) served as an abundant and ideal cell source for tissue engineering attracts interests of various researchers. The immunogenicity of MSCs, however, influences the security and efficiency of cell therapy. Our group aims to study the immune property of MSCs in a pro-inflammatory microenvironment (IE). We established the MSCs from hESCs, which were then exposed to IFN-γ. The major histocompatibility complex class I(MHC-I) expression on MSCs was up-regulated significantly in the IE. The MHC-I expression level of MSCs determined the proliferating rate of lymphocytes in one-way mixed lymphocytes culture and severity of delayed type hypersensitivity(DTH) in vivo. Besides, it affected the chondrocyte regeneration in an osteoarthritis milieu. The immune responses were strong in MSCs engrafted host. We concluded that the immunogenicity of ESC-MSCs increased under IE, which might increase the risk of immune rejection and reduce the therapeutic efficiency.

Session No.: Y04-06

Quiescent Preconditioned Human Multipotent Stromal Cells Adopt a Metabolic Profile Favorable for Enhanced Survival Under Ischemia

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A major impediment to the development of therapies with mesenchymal stem cells/multipotent stromal cells (MSC) is the poor survival and engraftment of MSCs at the site of injury. The ischemic environment (i.e. lack of both oxygen and nutrients) encountered by cells upon implantation is the prime cause of this cell death. We hypothesized that lowering the energetic demand of MSCs by driving them into a quiescent state would enhance their survival under ischemic conditions. Human MSCs were induced into quiescence by serum deprivation (SD) for 48h. Such preconditioned cells (SD-hMCs) exhibited reduced nucleotide and protein syntheses compared to unpreconditioned hMSCs. SD-hMSCs sustained their viability and their ATP levels upon exposure to severe, continuous, near-anoxia (0.1% O2) and total glucose depletion for up to 14 consecutive days in vitro, as they maintained their hMSC multipotential capabilities upon reperfusion. Most importantly, SD-hMSCs showed enhanced survival in vivo after implantation in an ischemic environment in mice for one week. Quiescence preconditioning modified the energy-metabolic profile of hMSCs: it suppressed energy-sensing mTOR signaling, stimulated autophagy, promoted a shift in bioenergetic metabolism from oxidative phosphorylation to glycolysis and up-regulated the expression of gluconeogenic enzymes, such as PEPCK. Rather than using a single pathway to ensure their survival under ischemia, SD-hMSCs likely use a multifaceted strategy to overcome this metabolic insult. The present investigation focused on the role of autophagy, the potential metabolic fuels, and on their related energetic pathways. Since the presence of pyruvate in cell culture media was critical for SD-hMSC survival, we speculate that these cells may utilize some steps of gluconeogenesis to overcome metabolic stress. These findings support that quiescence preconditioning causes a protective metabolic adaptation that might be taken advantage of to improve hMSC survival in ischemic environments.

Session No.: Y04-07

Atomized Mesenchymal Stromal Cells for Application in Lung Diseases

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Current treatment regimens for lung injuries are mainly supportive and rely on self-regeneration processes for recovery. Cell therapy with mesenchymal stromal cells (MSCs) is increasingly investigated and human amniotic mesenchymal stromal cells (hAMSCs) have potential use for lung regeneration. The aim of this study was to demonstrate that hAMSCs can be atomized for direct airway delivery and also to examine the influence of substrate stiffness and composition, representative of normal and diseased lung tissues, on hAMSC behavior. The study was designed to set the groundwork for future applications to healthy and diseased lungs for constructive tissue regeneration. To assess atomization and substrate effects, hAMSCs were sprayed using an LMA® MAD780 device onto either gelatin gel (10% w/v) or tissue culture plastic, as preliminary models that mimic stiffnesses of healthy and diseased lungs, respectively. Parallel effects of substrate composition on hAMSC viability and proliferation were by coating the tissue culture plastic with poly-L-lysine (PLL) and collagen I (rat tail) and by adding keratose (Merino wool) to the spray mixture. Delivery of atomized hMASCs was also assessed in decellularized rat lungs. The feasibility of atomizing hAMSCs was demonstrated with high cell viability unaffected by the different stiffnesses of the substrates (cell viability sprayed onto plastic or gelatin: 81 ± 3.1% and 79 ± 11.6%, respectively; control/non-sprayed: 85 ± 4.8%). Cells delivered by atomization to the three-dimensional lung models showed similar morphology with more uniform cell distribution compared to traditional instillation without spraying. Collagen I coating yielded higher proliferation and did not affect morphology of cells compared with both PLL coating and keratose supplementation. We demonstrated that hAMSCs maintain high viability when atomized onto substrates with different stiffness, especially in the presence of collagen. These results highlight the potential of the cell-spraying technology to enhance the effectiveness of cell therapy for lung regeneration.

Session No.: Y04-08

Aligned Mesenchymal Stem Cells Based Cell Sheet Engineering as a Novel Versatile Biological Tool in Tissue Regeneration

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The hallmark of cell sheet technology in creating both two dimensional and three dimensional scaffold-free tissue-like constructs has great synergy with transplant creation in tissue engineering. Recent focus in cell sheet technology utilizes the properties of mesenchymal stem cells (MSCs) which has the potential to self-renew and differentiate into multiple lineages when cultured in different environments. These properties of MSCs provide a unique advantage in stem cell-based therapy, offering versatility in usage. In this study, we integrate cell sheet technology, MSCs, the native structure and cellular alignment of tissues in the native environment to create a novel approaches for developing aligned MSCs based cell sheets for different aligned tissue reconstruction. We report the development of an aligned mesenchymal stem cells based cell sheet on a polydimethylsiloxane micropatterned surface which has the potential to be further developed into different aligned tissues (e.g. tendons and annulus fibrosus) by remolding them and subjecting them to different culture conditions. Our findings demonstrated upregulated tendon-specific markers and increased extracellular matrix production in a tendon-simulated condition, while upregulation of fibrocartilage-specific markers was demonstrated in AF-simulated condition. These results show the versatility in the development of this aligned MSCs based cell sheet, and extending and opening possibilities in tissue regeneration to generate tissues where cells/matrix alignment is pivotal such as those in muscles and blood vessels.

Session No.: Y04-09

Fabrication of Decellularized Adipose Tissue/Alginate Composite Microspheres with Pascs Encapsulation for Tissue Engineering

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Stem cells are utilized to create living and functional tissues to regenerate and repair tissue or organs in the body in cell based and regenerative treatments. It has been demonstrated that stem cell treatments can improve perfusion and stimulate neovascularization in peripheral arterial disease. However, such treatments usually suffer from some deficiencies, such as immune rejection and limited proliferation of implanted cells. In this study, we focused on the application of the decellularized adipose tissue (DAT), which is a native extracellular matrix (ECM), in cell based therapies. Biomaterials extracted from the organism itself possesses great potential as scaffold materials for stem cell culture as well as new tissue construct formation, especially if the ECM and cell are from the same source (in our case, the adipose tissue). Therefore, DAT solution (DATsol) was combined with alginate in our study to culture adipose stem cells (ASCs). We investigated the mixture of DATsol and high or low molecular weight alginate. The 2-D in vitro study of DATsol/alginate was found to promote cell adhesion and differentiation. ASCs, immobilized in DATsol/alginate microspheres, demonstrated metabolic activity which with an overall viability higher than 80%. The results suggested that the use of DATsol and alginate possesses great potential for application in cell based therapies in ischemia patients which facilitates the development of new mature and stable capillaries.

Session No.: Y04-10

Synergistic Targets Between ASC-differentiated Endothelial and Neural Lineage Cells to Prevent Hypoxic-ischemic Brain Injury

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Neonatal hypoxic-ischemic (HI) brain injury causes disruption of neurovascular integrity and leads to life-long functional deficit in human development. These devastating consequences can be prevented by protecting the architecture of the neurovascular unit. Our lab has demonstrated the possibility to differentiate the adipose-derived stem cells (ASCs) into endothelial lineage cells (ELCs) or neuronal lineage cells (NLCs) via microenvironmental induction. We studied the therapeutic effect of different cells by transplanting ASCs, ELCs, NLCs, or combination of ELCs and NLCs (E+N) into HI brain injured neonatal rats. As compared to the other treatment groups, combined E+N treatment showed a significantly greater decrease of infarcted and apoptotic areas with better preservation of tissue integrity. The E+N combinations increased the cell migration under in vitro hypoxia microenvironment, and the transplanted cells were able to engraft into host tissue and promote endogenous tissue regeneration. Moreover, we fish out the potential targets for the differentiated ELCs and NLCs. The ELCs migrated and contributed to the vascular structure by activating the Akt signaling through VEGF receptor 2 and neuropilin 1 (NRP1). NLCs showed better ability in preserving the neural structure. The synergistic benefits in E+N combination were revealed via the cell-cell interactions for NRP1 signaling in ELCs and C-X-C chemokine receptor 4 (CXCR4) and fibroblast growth factor receptor 1 (FGFR1) signaling in NLCs. Inhibiting of target signals in either ELCs or NLCs diminished the synergistic interaction in cell migration, homing and protective effect. Using the E+N treatment with blockage of NRP1 signaling in ELCs or either CXCR4 or FGFR1 signaling in NLCs significantly diminished the beneficial effect on preserving tissue integrity after HI injury. Current study emphasized the role of microenvironmental interaction via potential molecular targets in the synergistic effect of combined ELCs and NLCs on improving both structural and functional outcome after HI injury.

Session No.: Y05-01

Hgf/Heparin-immobilized Decellularized Liver Matrix as Substrates for Hepatocytes Regeneration in Acute Liver Injury Model

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The liver is one of the most important organs in the human body. However, liver failure has always been a serious threat to millions of people’ s health. Such loss of organs and tissues can be effectively treated by skilled transplantation or partial hepatectomy (PH) treatment. Nevertheless, transplantation is limited by a critical donor shortage. Besides, the risks of healing process after PH treatment are also the problem. Therefore, an HGF/heparin-immobilized decellularized liver matrix (HGF/heparin-immobilized DLM) was developed for hepatocytes regeneration in acute D-galactosamine-induced liver injury. In the result, (1) the amounts of immobilized heparin on DLM were 4.7± 1.1, 12.2± 2.7, 18.2±5.0, 21.9±3.8, 29.1±1.1 μg/cm2 when the initial heparin concentrations were 0.2, 0.4, 0.6，0.8 and 1 g/L, respectively (2) The mitochondria activity and albumin synthesis of the hepatocytes on HGF/heparin-immobilized DLM film was 10-30% and about 10% superior than in normal dish at 3 days of culture, separately. (3) The lactate dehydrogenase activity of the D-galactosamine-induced injury of hepatocytes on HGF/heparin-immobilized DLM film cultures was 10 milliunits/mL, which is 50% lower than that in the D-galactosamine-induced injury of hepatocytes on normal dish cultures. (4) The DNA quantity of D-galactosamine-induced injury of hepatocytes on HGF/heparin-immobilized DLM film cultures was 30-50% more than in the D-galactosamine-induced injury of hepatocytes on normal dish cultures. In summary, HGF/Heparin-immobilized DLM showed higher potential in hepatocyte culture and also repaired injured hepatocytes from D-galactosamine. It is believed that this heparin-immobilized DLM film has promising potential for hepatocyte transplantation, and could be applied for future liver tissue engineering use.

Session No.: Y05-02

Fabrication and Delivery of Structurally Controlled Microtissue by Using Micropatterned Thermo-sensitive Hydrogels

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Native tissue shows unique micro-structure which is closely related to its shape and functions. Various techniques based on microfabrication have been developed to mimic natural tissues with structural characteristics including bio-MEMS, patterned array and microfabricated scaffolds, which have been used in bioassay or therapy. However, it is hard to harvest and to directly deliver of pre-organized cells with arranged structure to targets without the use of additional support while maintaining extracellular matrix molecules (ECM). In this study, we prepared structurally regulated microtissue from human dermal fibroblasts (HDFBs) on a thermally expandable hydrogel via micro-contact print (μ-CP) of polydopamine (PD). First, we fabricated polydimethylsiloxane (PDMS) stamp with 50, 100, and 200 μm stripe patterned groove in width. Each grooved PDMS stamp was coated with PD, which was then used for μ-CP on thermo-sensitive hydrogel to induce regulated cell adhesion. After seeding of HDFBs, cells were well attached and aligned along the direction of printed patterns on hydrogel forming string structure. Well arranged cell strings were delivered to target by expansion properties of thermo-sensitive hydrogel. The prepared microtissue maintained their viability and aligned structure in defined area on target. Finally, we could obtain free-standing cell string which was forming bridge-like structure between PDMS supports. Therefore, our results showed that advanced cell structure delivery system can be an effective method for constructing and harvesting microtissue with complex structures.

Session No.: Y05-03

Polypyrrole and Laminin Coated Pmma Nanofiber Scaffold for Skeletal Muscle Tissue Engineering

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Polypyrrole and laminin coated PMMA nanofiber scaffold for skeletal muscle tissue engineering Kamalia Z1, BHI Ruszymah1,2, SR Chowdhury1 1Tissue Engineering Centre, Universiti Kebangsaan Malaysia Medical Centre, Malaysia 2Department of Physiology, Faculty of Medicine, Universiti Kebangsaan Malaysia Skeletal muscle mainly contains contractile myoblasts and non-contractile fibroblasts. The extracellular matrix (ECM) microenvironment of muscle strictly regulates the population of these cells for desired tissue function. Electro-conductivity is another unique feature of muscle tissue that requires for its contractile activity. To resemble the native tissue, we fabricated electrospun polymethylmethacrylate (PMMA) nanofibers coated with laminin and polypyrrole (PPy) (PMMA-Lam-PPy) for skeletal muscle tissue engineering. In this construct, PMMA nanofibers coated with laminin mimics the ECM microenvironment for skeletal muscle tissue, while PPy contributes electro-conductivity. PMMA nanofibers were fabricated by electrospun technique and coated with laminin (50g/ml) and polypyrrole (0.25%). Scanning electron microscopy analysis demonstrated that average diameter of PMMA nanofibers was approximately 380 nm. Coating with PPy and laminin marginally increase the fibre diameters, however, no significant difference was observed. Fourier transform infra-red analysis confirms the coating of PPy and laminin on the PMMA nanofibers scaffold. Four-point probe analysis revealed that the conductivity of the PMMA-Lam-PPy scaffold was approximately 4.5 s/cm2. Consequently, myoblasts and fibroblasts, isolated from skeletal muscle tissue of three consented patients, were co-cultured on PMMA-Lam-PPy scaffold. It was found that myoblast population increased by 20% during expansion on PMMA-Lam and PMMA-Lam-PPy due to the increase in myoblast growth rate than fibroblasts. Whereas, no changes in myoblast populations were observed on plain and PMMA nanofibers. These result suggested that coating with laminin and PPy produces a conductive scaffold which facilitates myoblast proliferation, thus, it could be a promising scaffold for muscle tissue engineering.

Keywords: Muscle tissue engineering, nanofibers scaffold, polypyrrole, laminin, myoblast, fibroblast

Session No.: Y05-04

Synthesis, Characterization and Application of Photocrosslinkable Biodegradable Elastomer PGSA

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The development of biodegradable materials with elastomeric properties had become one of the most popular research topics in the past decade, and the need to produce new elastomeric polymers in large scale for a wide variety of applications had been ever increasing. Poly(glycerol sebacate) (PGS) is a novel elastomer, with high biocompatibility and biodegradability, and has been applied in studies for soft tissue regeneration with promising results. However, the fabrication of PGS requires high temperature and low pressure, thus limiting its devicing capability and applicability in medicine and tissue engineering. With this need, the novel photocrosslinkable polymer, poly(glycerol sebacate) acrylate (PGSA) was synthesized. A wide range of mechanical properties are obtained with respect to their Young's modulus from 0.12 to 3.17 MPa, ultimate tensile strength between 0.1 and 1.2 MPa and strain to failure between 39% and 121%by changing the degree of acrylation. Linearly degradation properties are observed and are degraded between 8.5 to 28% in 30 days when decreasing degree of acrylation. A series of cell culture were conducted for the confirmation of biocompatibility along the purification of cured films. In this work, we focus on the fabrication and application of a photocurable, biocompatible and biodegradable polymer that can be safely used in tissue engineering and biomedical engineering.

Session No.: Y05-05

Bi-perfusion System Design Applied to Fabrication of Porous Chitosan-gelatin Liver Scaffolds

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Whole liver transplantation is still a highly successful cure for end-stage liver failure nowadays, although the transplantation is limited by a critical donor shortage. Due to a remarkable capacity to regenerate, liver can re-grow within a week after the partial hepatectomy (PH); however, the risks of complications and surgical morbidity which reduce the feasibility should be carefully evaluated. One of the risks is liver cirrhosis, caused by the ineffective tissue perfusion and the change of environmental pressure, and is an index for PH. For this reason, sufficient tissue perfusion and oxygenation are essential for all cell and tissue metabolism that is the key to successful tissue repair. As a result, this study placed the focus on efficient liver scaffolds. Instead of liver transplantation and PH, the Bi-perfusion system design in Chitosan-Gelatin (C/G) liver scaffolds were developed as novel clinic method for recovering the failure liver tissue. C/G liver scaffolds with Bi-perfusion system were manufactured by using uniform fabrication and freeze-drying techniques. Predesigned geometry arrows inside C/G liver scaffolds have the following advantages in hepatocyte culture: (1) to enhance hepatocyte viability by establishing a Bi-perfusion system; (2) to increase the nutrient supply to hepatocyte by altering the matrix distance limit in the Bi-perfusion system; (3) to confirm the correlation between the structure strength and hepatocyte morphology in the rheological simulations multi-coupling analysis experiment. In the conclusion, the implant of C/G liver scaffolds, in place of whole liver transplantation, might provide a method to engineer new liver tissue and recover the failure tissue that will treat many patients in the next generation. This system may have the potential for liver tissue engineering in the future.

Session No.: Y05-06

Engineering Perfusable Vessel-like Constructs with Multilayer-hydrogels and Cells Based on 3D Printing

Juan Liu[1], Hsi-Wen Chen[1], Zhifen Chen[1], Shicheng Sun[1], Sascha Schwarz[2], Huaiyuan Zheng[1], Patrina Poh[1], Hans-Günther Machens[1], Arndt Schilling[1]

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Engineering perfusable vessel-like constructs with multilayer-hydrogels and cells based on 3D printing Juan Liu1, Hsi-wen Chen1, Zhifen Chen2, Shicheng Sun2, Sascha Schwarz3, Huaiyuan Zheng1, Patrina S.P.Poh4, Hans-Günther Machens1, Arndt F.Schilling1,3 1. Lab of Plastic Surgery and Hand Surgery, Klinikum rechts der Isar, Technische Universität München 2. I. Medical Department - Cardiology, Klinikum rechts der Isar,Technische Universität München 3. Center for Applied New Technologies in Engineering for Regenerative Medicine(Canter), Munich, Germany 4. Lab of Traumatology , Klinikum rechts der Isar, Technische Universität München The principle of tissue engineering is to create a three-dimensional(3D) scaffold embedded with functional cells, which could be transplanted into human body and promote tissue and organ regeneration. However, the size of engineered tissue constructs is mainly constrained by diffusion limitation. In any tissue constructs, it is essential for embedded cells to get enough oxygen, nutrition as well as optimal growth factors in order to maintain their viability and functionality. Therefore, fabrication of perfusable vessel-like constructs mimicking natural structures and intergration of them within engineered tissue constructs is a prerequisite for engineering real functional tissues for regeneration and novel biomedical applications. This study demonstrated a fast and economical way of engineering multilayer biomimetic vessel-like constructs with multi-cell types based on 3D printing. Multilayer vessel-like constructs, which physiologically mimicked the natural structures of vascular wall, were fabricated with cell-laden fibrin gels in 3D-customized PDMS bioreactors. This bioreactor enables in situ fabrication of cell-laden hydrogels and continuous dynamic perfusion for maturation of vessel-like structures. Based on 3D printing technique, length of constructs, thickness of each layer, diameter of inner lumen and the whole vessel constructs were easily designed and controlled. The multilayer vessel-like constructs could potentially be used in biomedical applications including being embedded into more complex tissue constructs for vascularization, study of vascular biology, drug screening, and developing in vitro models for cancer metastasis.

Session No.: Y05-07

Novel Biomaterial for Regenerative Medicine Based on Human Mesenchymal Stromal Cell Secreted Products

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Tissue engineering is a fast growing area of regenerative medicine aiming at structural and functional regeneration or replacement of damaged tissues and organs. Human mesenchymal stem/stromal cells (MSC) combined with different kinds of scaffolds are often used for tissue engineering. MSC produce many bioactive factors involved in multiple reparative and regenerative processes in damaged tissues. MSC conditioned medium (MSC-CM), contained a combination of various secreted components produced by MSC, could mediate most of beneficial regenerative effects of MSC and lack possible side-effects of using MSC themselves. We have developed a prototype of a novel biomaterial for regenerative medicine based on the combination of MSC-CM with different types of collagen scaffolds. We have suggested the optimized protocol of human adipose-derived MSC-CM production based on the dynamics of key growth factors secreted by cells during long-term conditioning in clinically compliant media. MSC-CM demonstrated specific activity in vitro by stimulating the migration of human fibroblasts and endothelial cells, which are both important for damaged tissue reparation and regeneration. We have combined MSC-CM both with collagen gel to produce a biomaterial for injection and with collagen wound dressing. We have revealed an optimal collagen/CM relation to manufacture scaffolds with appropriate mechanical structure and MSC secreted factors release. The regenerative effects of the manufactured constructs were successfully tested in vitro and in vivo for wound healing and spermatogenesis recovery. The developed biomaterials could be further improved to enhance their therapeutic activity and produce new modes for various clinical applications.

Session No.: Y05-08

Coating of Monoclonal Antibodies Anti-integrin β1 and R-Phycoerythrin-preconjugated Anti-CD73 on Bovine Serum Albumin Matrixes by Two-photon Crosslinking

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Coating of Monoclonal Antibodies Anti-integrin β1 and R-phycoerythrin-preconjugated Anti-CD73 on Bovine Serum Albumin Matrixes by Two-Photon Crosslinking Chi Hung Yip, Nan Huang, Barbara Pui Chan Tissue Engineering Laboratory, Department of Mechanical Engineering, The University of Hong Kong, Pokfulam Road, Hong Kong Special Administrative Region Objectives: For the characterization of the less-readily available healthy autologous cells in tissue engineering, micro-scale characterization is more favorable than conventional approaches like flow cytometry, due to the reduction in invasive harvests and donor site injuries. Multi-photon bio-fabrication is an emerging technology to immobilize natural biological molecules with ultra-high spatial resolution without the use of harsh materials and conditions. We have previously demonstrated the controllability in various mechanical properties, porosity, and the extracellular matrix. In order to further extend the current technology capabilities to micro-scale cell characterization, this study aims to immobilize antibodies (anti-integrin β1 and R-phycoerythrin(PE)-preconjugated anti-CD73) by two-photon photochemical crosslinking and investigate into the dose-, power- and scan cycle-dependence. Methods: Bovine serum albumin (BSA) was first photo-crosslinked to form rectangular matrixes of 101μm x 101μm x 5μm, in the presence of photosensitizer Rose Bengal (RB), by a femto-second laser at 800nm wavelength and 150mW power. Antibody/RB mixture was loaded after washing the construct with phosphate-buffered saline. Square patterns of 20μm x 20μm were coated onto the matrixes by photochemical reactions at different powers and scan cycles. The antibody immobilization efficacy was evaluated by immunofluorescent staining. Results: Anti-integrin β1 and PE-preconjugated anti-CD73 at different concentrations were immobilized and patterned onto BSA matrixes. The dose- , power- and scan cycle dependency of the efficacy were examined. Cellular binding to the antibodies are underway. Conclusion: This study presents the use of multiphoton photochemistry for spatially controllable antibody immobilization. The novel platform with high-resolution spatial control and multiple controlling parameters provides additional freedoms in designs and optimizations.

Session No.: Y05-09

Biomimetic Whitlockite Nanoparticles-mediated in Situ Bone Regeneration

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Living bone self-repairs micro-damaged sites via creating local acidic microenvironments for initial resorption followed by sequential new bone formation. Despite previous achievements in understanding sophisticated signaling pathways between cells and bone extracellular matrices during bone remodeling process, a role of local ionic concentration during bone regeneration remains to be elucidated. Here, we demonstrate that synthetic whitlockite (WH: Ca18Mg2(HPO4)2(PO4)12) nanoparticles can recapitulate early-stage bone regeneration by increased local ionic concentration and subsequent neo-bone formation. Our studies show that WH promotes osteogenic differentiation of stem cells by continuous supply of PO43- and Mg2+ under physiological conditions. In addition, unique physiochemical property of WH surface promotes protein adsorption, facilitating inorganic-protein interactions. We also demonstrate that a biomimetic WH-embedded cryogel can regenerate complete bone tissues. Collectively, these findings identified feedback interactions suggesting that the WH nanoparticle-based cryogel platform may contribute to promoting cell recruitment1 and bone recovery via PO43- and Mg2+ channels induced metabolic pathway.

Session No.: Y05-10

Tissue Engineered Bone for the Biomimetic Capture of Bone Metastatic Cancer Cells

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[2]Southwest University

Bone metastasis is a significant source of mortality and morbidity in late stage cancers of prostate, breast and lung. The bone marrow stromal niche, in particular, has been established as the preferred 'soil' for metastatic seeding by prostate cancer (PCa). However, this metastatic process remains poorly understood due to the lack of appropriate experimental disease models. This problem is exacerbated by the inability of animal models to reliably develop skeletal metastasis and further hindered by the difficulty to examine the process at single cell level. Previously, several groups, including ours, have observed the invasion of PCa cells into xenotransplanted human bone grafts in an immunocompromised mouse model. Here, we propose the use of similar engineered bone microenvironments (BM) on microfluidic devices to elicit the capture of osteotrophic cancer cells. These captured cells may subsequently be used for in situ assessment of phenotypic changes or be extracted for further molecular analysis. Microfluidic devices were fabricated using lithography methods, comprising three channels housing mesenchymal stromal cells (MSC) and cancer cells (CC), separated by a collagen gel-filled capture region respectively. Bone metastatic PCa cells (PC3) were loaded into CC channel and migration was observed over a period of four days. Simulated bone marrow environment was found to induce migration of PC3 cells into the capture region (MSC group: 70.7±28.9 PC3, 48.6±47.8µm; Control: 10.7±13.6 PC3, 13.5±10.8µm). This effect was abolished by the addition of AMD3100, indicating a role of CXCL12-CXCR4 axis in PC3 migration. MSC spheroids were deployed in the device to allow for recapitulation of the native 3D microenvironment, resulting in increased PC3 migration. Taken together, our results demonstrated the effectiveness of our device to successfully sequester bone metastatic cancer cells, which may be used as an experimental model of early metastatic events and facilitate research on metastasis.

Session No.: Y06-01

The Effect of Extrusion-based Printing Parameters on 3D Resolution: A Systematic Study

Ting Guo[1], Timothy Holzberg[1], Casey Lim[1], Ankit Gargava[1], Srinivasa Raghavan[1], John Fisher[1], Timothy Holzberg[1], Casey Lim[1], Ankit Gargava[1], Srinivasa Raghavan[1], John Fisher[1]

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With the development of computer-aided design techniques and the understanding of biomaterials, 3D printing has become a popular and advantageous way to produce scaffolds with complex, patient-specific structures. While many different materials have been assessed for 3D printing, due to the lack of systematic research on the printing procedures, the reported fabrications are specific and case dependent. Our present study utilizes advanced evaluation process incorporating micro CT scanning and computational statistic modeling to investigate the correlation between printing parameters and the resulting printing resolution. The study uses poly(lactic-co-glycolic acid) (PLGA) as the printing resin and an extrusion-based additive manufacturing to fabricate the scaffolds. Five different types of PLGA with various molecular weight, lactic acid (LA) to glycolic acid (GA) ratio and end cap were chosen to investigate the dependence of the extrusion process on the polymer composition. We determined the material rheology and thermal status range that are suitable for high quality print by relating the material properties to the printing output. We observed that PLGA with higher LA:GA ratio and an ester end cap showed superior consistency in terms of printing resolution as a result of better material thermal stability. The printed scaffolds with different combinations of formula, patterns, and printing conditions were evaluated using microCT. A statistical model, which analyzed the relationship between resolution (interpreted by actual fiber diameter and spacing) and fabrication parameters, revealed that temperature and speed are the dominant factors to consider for the extrusion printing resolution while the impact of needle diameter and printing pattern is moderate. We also showed that the extruded fibers encouraged cell alignment along the printed fibers. The well-characterized PLGA scaffolds with high resolution can be used in many tissue engineering applications such as osteochondral defects repair. This systematic study will contribute in developing standards for the 3D bioprinting.

Session No.: Y06-02

Drop-on-demand Printing of Polyvinylpyrrolidone-based Cellular Droplets with Improved Printing Consistency and Cellular Viability

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Bioprinting is an emerging research field that has recently attracted tremendous attention; it offers a highly automated, advanced manufacturing platform for fabrication of complex bioengineered constructs comprising additional cell types and biomaterials to improve the homology to native tissues and/or organs. In native tissues, cells are spatially patterned at high degree of specificity within a three-dimensional (3D) space to allow an intricately orchestrated exchange of signals that regulate their migration, proliferation, differentiation. Particularly, the drop-on-demand (DOD) bioprinting enables higher degree of control over the cellular densities and positioning. However, the use of cell-encapsulated hydrogels as printable bio-inks sometimes hinder control over cellular densities and positioning within the bioprinted constructs due to the random distribution of encapsulated cells within the hydrogels. This work aims to investigate the effect of polyvinylpyrrolidone (PVP) on the composition of cell-laden hydrogel for optimised DOD printing. It has been reported that PVP has an influential role in accelerating tissue maturation in a dose-dependent manner; hence this work pioneers the novel use of PVP-based bioinks in 3D bioprinting. A suitable range of PVP concentrations (0-3% w/v) for our microvalve-based (DOD) bioprinting system was first evaluated; following which the effects of PVP-based bio-inks on the printing output consistency and cellular viability of fibroblasts (ATCC® SCRC-1041TM) were characterized. Our findings highlighted that significant improvements in both the printing output consistency (1.24 ± 0.91 in 0.5 mil cells/ml, 3.00 ± 1.06 in 1.0 mil cells/ml, 5.22 ± 1.18 in 1.5 mil cells/ml and 7.58 ± 1.63 in 2.0 mil cells/ml) and cellular viability (> 95%) were observed in the PVP-based bio-inks, suggesting that a more viscous bio-ink (~ 8 mPa.s) is favourable for bioprinting applications.

Session No.: Y06-03

Development of 3D-plotted Polycaprolactone/Calcium Silicate Scaffolds Coated with Decellularized Extracellular Matrix for Bone Tissue Regeneration

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Regenerative medicine has been a promising method in restoring malfunctioning cells, tissues, organs through the usage of cells incorporated in bio-scaffolds. In these approaches, newly undifferentiated stem cells are planted at the site of pathologic lesion so that the stem cells can eventually differentiate into the desired phenotypic form. The extent of cellular growth and differentiation has been enhanced by incorporating the stem cells into biocompatible scaffolds to ensure mechanical stability during regeneration processes. Polycaprolactone (PCL) and calcium silicate (CS) biomaterials are proven to have therapeutic characteristics for bone tissue regeneration and can be used to manufacture bio-scaffolds. However, these synthetic scaffolds cannot fully mimic the complexity of the extracellular microenvironment. Extracellular matrix (ECM) comprises of numerous essential growth factors and structural proteins that sustains optimal cellular growth and differentiation. Decellularization of ECM could be a possible solution to this problem as this process removes nearly all the genetic molecules of the cells but simultaneously retains the crucial growth-supporting proteins inside the ECM. In this study, decellularization processes were performed on MG-63 (human osteosarcoma cell line) and human umbilical vein endothelial cells (HUVECs) and incorporated into the 3D plotted biocompatible PCL and PCL/CS scaffolds. These decellularized ECM (dECM) bio-scaffolds are promising complexes that can support essential bone tissue regeneration processes, including osteogenesis and angiogenesis, by mimicking the optimal microenvironment for cell growth and differentiation. Wharton's jelly mesenchymal stem cells (WJMSC) are planted into these dECM scaffolds and the extent of cell differentiation and proliferation were examined.

Session No.: Y06-04

Development of Observing Dispersion of Hydroxyapatite in Polymer Matrix with Electron Microscopy

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Poly(1,6-bis-(p-carboxyphenoxy hexane)-co-(sebacic anhydride)) (PANH), is a polyanhydride copolymer which has good biocompatibility, and degrades to non-toxic products with a predictable rate of degradation. Nano-sized hydroxyapatite (HAP) is a well-known biomaterial which has been applied in bone regeneration due to its osteoconductivity. But it has poor colloidal stability which leads to agglomeration when incorporated into polymeric composites. In this work we describe the surface grafting of poly(ε-caprolactone) to HAP (PCL-gHAP) to improve the dispersion of HAP particles in a PANH matrix to form a composite material. However, the difference of the stiffness between polymer matrix and fillers make it difficult on sample preparation for electron microscopy imaging. We develop the use of scanning electron microscopy-backscattered electron (SEM-BSE) detector base on the different element in polymer and HAP generates different signal intensity. The technique of focused ion beam (FIB) is an ideal tool for preparation of TEM specimens as it allows the fabrication of electron-transparent foils with a typical thickness of 150 nm from any region of interest. FIB works by sputtering atoms from the target material by bombarding the target with accelerated heavy ions. Also, we demonstrate the combination of FIB technique and BSE images to build up the 3D nano-structure of the composite. These images show that surface modification of HAP with PCL improved the homogeneity of the dispersion of HAP particles in the composites. The developments show a powerful approach on imaging with maintaining the nano-structure of composite samples.

Session No.: Y06-05

Investigation Integrin-FAK-Src Intracellular Signaling for Artery and Vein in Response to Shear Stress

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Mechanical environment and shear stress play essential role in cardiovascular system for endothelial cells (ECs) homeostasis, proliferation, apoptosis, and functional expression. How the cell senses mechanical stimulation and convert it into cellular mechanobiological signal cascades for different cell function is still not well understood. Cells can attach to the extracellular matrix (ECM) by focal adhesions (FAs) using focal adhesion kinase (FAK) and integrins. Phosphorylation of FAK (p-FAK) activates Src and initiates intracellular signal cascade for signal transduction. The purpose of this study is to compare the difference response of ECs from artery and vein to response the arterial laminar shear stress (ALSS). The GFP-FAK and RFP-F-actin were co-transfected and used the time-lapse confocal microscopy to monitor the dynamics of FAK and stress fiber in single cell. FAK localized at peripheral of cell membrane and linked with the end of stress fiber. When the arterial ECs were subjected to ALSS , cell occurred elongation morphology and were parallel to LSS direction. However, the venous ECs showed decrease of FAK assembling and may peer off under ALSS. When apply the FI14 to inhibit FAK and cell migration in arterial EC, the flow response remodeling in FAK and stress fiber was suppressed under both static and flow condition. Therefore, FA and cytoskeleton dynamic are important for EC remodeling in response to the shear stress

Session No.: Y06-06

Bone Marrow On-a-chip for One-step Recruitment and Expansion of Leukemic Stem Cells

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Leukemic Stem Cells (LSC), which show intimate interactions with the bone marrow (BM) niche, are known to be responsible for drug resistance and relapse. To date, however, there is a lack of adequate markers to identify bona fide LSC. This raises the importance to study these LSC in more detail to develop more effective disease models and therapies. Here, we propose the use of a microfluidic device to artificially engineer bone marrow environment to chemoattract true LSC populations. Polydimethylsiloxane is used to fabricate these devices using soft lithography techniques. The device comprises two side channels, housing leukemic cells and BMSC respectively, separated by a collagen matrix capture channel. Bone marrow stromal cells (BMSC) are used to mimic the BM niche while THP-1 cells are used to model acute myeloid leukemia. THP-1 will migrate towards the BM niche in the co-culture system, which can then be quantified and extracted for further analysis. Our findings demonstrate that the device supports co-culture of BMSC and THP-1 with over 90% viable cells at 72h post-seeding. BM was able to recruit a sub-population of THP-1, resulting in an increased number of cells captured. 129±29 cells migrated towards the BM niche as compared to 0 cells in control setup at 72h while the distance migrated towards the BM niche was 61.7±51.4μm compared to 0 μm in control setup at 72h. The addition of AMD3100 CXCR4 antagonist attenuated these effects in a dose dependent fashion, mirroring clinical observations that leukemic migration to bone marrow is induced by CXCR4-SDF1 axis. Fluid flow was subsequently introduced to represent in vivo flow and shear stress conditions in order to achieve a more physiologically relevant model. These studies provide a proof-of-concept for using this device to study the LSC subpopulations in greater detail.

Session No.: Y06-07

Fabrication of Functional Hepatic Tissue by Cell Sheet Technology in Vitro

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Liver plays a significant role in a human body not only in secreting important hormone such as insulin-like growth factor and bile but also metabolizes glucose, lipid and proteins. Another crucial function of the liver is to metabolize drugs. Hence, in-vitro hepatocyte based drug screening is vital for the growing pharmaceutical studies in evaluation of efficacy and toxicity of new and improving drugs. However, the challenge working with hepatocyte rises as it tends to lose its morphological characteristic and biological function rapidly in-vitro. Various approach to maintain hepatic function have been and are still being studied, and our laboratory with the advantage of cell sheet engineering technology successfully fabricated hepatocyte cell sheet. This technology holds a potential to manufacture tissue that imitates in-vivo liver structure simply by layering hepatic cell sheets. The objective of this study is to fabricate functional hepatic tissue in in-vitro utilizing cell sheet technology. Co-cultured cell sheets composed of sinusoidal endothelial cells and hepatocytes from rat liver were fabricated to assist hepatic tissue production. Monolayer hepatocyte cell sheet was sandwiched between two co-culture cell sheets and cultivated in static culture. For biological evaluation, albumin secretion was measured. The albumin secretion for conventional culture decreases gradually for the observation period of two weeks while the same function increases for multi-layered co-culture sheets. Cryopreserved primary human hepatocytes cells were harvested in order to fabricate functional human hepatic tissue and albumin secretion and urea synthesis were measured to understand their function. From the evaluation, albumin secretion and urea synthesis in human hepatocyte cell sheets group were higher than conventional cell culture. Thus this suggested that cell sheet technology is capable in fabricating functional hepatic tissue.

Session No.: Y06-08

Development of Biodegradable and Conductive Film Incorporated with Photothermal Nanoparticles for the Control of NSC Differentiation

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Transplantation of differentiated human neural stem cells (hNSCs) into the injured site of central nervous system (CNS) may provide therapeutic benefit. It has been reported that electrical conducting substrate promoted human neural stem cells adhesion and their differentiation into neurons. Thus, in this study, an electrical conductive film composed of oxidative polymerized phenyl/carboxyl-capped aniline pentamer (CCAP) and ring-opening polymerized tetra poly(D, L-lactide) (4a-PDLLA) wasdeveloped. In addition, nanoparticles based on polyaniline (PAni) and polyoxyethylene-stearate were embedded in the film as a photothermal (PT) agent. This conductive film was suggested to act as a substrate for endogenous electric fields transmission in tissue, resulting in the improvement of tissue regeneration and wound healing. To accelerate the regeneration process, the PAni NPs triggered by near-infrared were used as a heat source to create a mild heat environment. In the results of 1H NMR, CCAP and 4a-PDLLA were successfully prepared and characterized. The coupling of CCAP and 4a-PDLLA was confirmed by FT-IR. According to cyclic voltammetry and UV-visible spectrum, the transition between different oxidation state and doping/ dedoping process of CCAP were achieved. PAni NPs showed excellent photothermal conversion efficiency under NIR with wavelength of 808 nm. In order to differentiate hNSCs into neurons, the cell response to electrical and thermal stimulus will be demonstrated.

Session No.: Y06-09

The Role of Excessive Autophagy Formation to Damage the Endothelium of Vein Graft

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Little is known about the interrelations between autophagy and endothelial damage in the pathological progression of vein graft disease. The venous-arterial transition induced excessive autophagy to trigger the signal cascades for the apoptosis of venous endothelial cells (ECs) and leaded to neointimal hyperplasia. We also discovered the pretreatment of vien with autophagy inhibitor prior the grafting surgery provides a good therapuetic strategy to modulate local autophagy activity. However, the detail mechanism of autophagy formation in the pathological progression of vein graft disease still remains unknown. To mimic the vein graft disease induced by mechanical overload, we artificially induced excessive autophagy in ECs via inhibiting of mTOR-relative signals in mTORC1 and mTORC2 (such as rapamycin, Torin1 and PP242) for 24 hrs. By assessing the autophagic protein expressions using western blotting, the decrease of p62 and increase of both LC3 and beclin1 indicated the autophagy induction in ECs under static condition. With high dosage of Torin1 and PP242, we observed the cell damage and expression of cleaved-PARP suggested that the excessive autophagy may induce ECs damage. The ECs with different modulation level of autophagy were then subjected to shear stress to mimic the clinical pathogenesis. This study provides further knowledge for the dynamics of autophagy formation in endothelium remodeling.

Session No.: Y06-10

Spatially Patterned Virus Patch for in Vivo Gene Delievery

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Gene therapy holds the significance of correcting genetic defects. However, difficulties in the in vivo delivery to the targeted tissues, and the systemic delivery remains to be the biggest challenge to be overcome. Here, a robust system of biofunctionalized polymeric layer-mediated lentiviral delivery was designed for site-specific spatial and temporal control of viral gene delivery. Through poly glycidyl methacrylate (pGMA) mofiication of a substrate via initiated chemical vapor deposition (iCVD) followed by polyethylenimine (PEI) immobilization provided the adhesion site for lentivirus. Furthermore, the polymeric patch based gene delivery system showed a high rate of gene transfection compared to bolus transfection. Furthermore, utilizing mask patterning, we were able to spatially patten the lentivirus which allowed spatially defined transfection.

Session No.: Y07-01

Development of Three-dimensional Cardiac Tissue Construct Using Decellularized Gastrointestine and Cell Sheet Technology

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Engineering three-dimensional tissues in vitro are known to be difficult due to the lack of vascularization. The purpose of this study is to create 3-D myocardial tissues by layering cell sheet in vitro and implanting the tissue as a new cure for heart failure. Tissues in living bodies have minute vascular networks by which oxygen and nutrients are supplied to tissues continuously. If these supplies were to be cut off, tissues will necrotize as a result of lack of oxygen and nutrients. Therefore, creating a vascular bed which is used by guiding perfusion in the construction of the tissue in vitro is important. We aim to construct an implantable vascular bed by recellularizing acellular rat's and pig's intestine with human endothelial cells. We investigated on how to decellularize and recellularize the tissues. To create a transplantable vascular bed, we used a part of rat's intestine with a loop between artery and vein. After harvesting the tissue (φ = 7 mm, L= 20 mm), both the lumen of intestine and vascular network were perfused continuously with deionized water at 4℃ for 24 hours, DCA at 25℃ for 4 hours, and DNase-I at 37℃ for 3 hours by using a roller pump to ensure the rate of 25 μL/min. After decellularization, the tissue was analyzed by hematoxylin and eosin staining, immunostaining and amount of DNA were calculated to confirm that the tissue meets the criteria for a decellularized tissue. Furthermore, after human endothelial cells were seeded onto the decellularized tissue and cultured for one week, it was confirmed that endothelial cells forms monolayer cell network in the vascular branches. In the future, investigation on the decellularizing and re-endothealizing tissue using pig intestine which will be used for clinical application will be done.

Session No.: Y07-02

Liver Regeneration Using 3D Printed Glycerol-based Biodegradable Scaffold

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Liver diseases have been the highest cause of death for many Asian countries, resulting in increasing need for liver transplant. As liver transplant becomes more common, the success rate of transplantation surgery had increased dramatically within the past two decades. However, post-surgery infection and side effects from immunosupressants remain a major cause for post-surgery death in patients. This work focuses on the development of a three-dimensional scaffold via additive manufacturing of biodegradable polymeric material for liver regeneration. The novel technology will provide an alternative toward MEMS-Fab, one technology that is precise, yet extremely expensive and time consuming. In this work, various 3D porous scaffolds are developed for hepatic cell growth via 3D printing using a novel glycerol-based material. In a wide range of 3D geometric design, hepatic cells are expected to respond differently through proliferation, elongation, and migration. Here, three porous structures are fabricated and seeded with hepatocytes: squared, circular, and hexagonal pores, ranging between 100 and 300 micrometer in diameters. Scaffolds of various mechanical properties are also cultured for optimal cell growth. Utilizing the high flexibility of 3D printing, full liver regeneration with vasculature and hepatocyte co-culture is considered the next step of this work.

Session No.: Y07-03

Spontaneous Self-organizing Molecule with Angiogenic Peptide as Biofunctionalized Hydrogel for Central Nervous System Regeneration

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Brain injury is a devastating medical condition that affects more than 10 million individuals annually worldwide and represents a major health problem. For such disease, tissue and organ regeneration has been regarded as an optimal therapeutic strategy. In this study, we proposed a regenerative strategy focusing on the provision of peptide-based three-dimensional matrix to facilitate neoangiogenesis around damaged brain wound region. A functionalized peptide, RADA16-SVVYGLR (AcN-RADARADARADARADASVVYGLR-CONH2), is designed, synthesized, and self-assembled into beta sheet secondary structure to form hydrogel with nanofibers network entanglements. The hydrogel with mechanical stiffness provides a suitable substrate for encapsulated neural stem cells adhesion and differentiation. Furthermore, the functional peptide sequence, SVVYGLR, was derived from osteopontin and had been shown to regulate growth of hemopoietic stem cells and to induce angiogenesis. From physical properties examination including nanofibers morphology and mechanical stiffness, the results showed pH- and ion- dependent changes. When pH value was adjusted from acidic to basic environment with ion addition, the nanofibers transformed into globular aggregates resulting in hundredfold stiffness increase. By previous in vitro and in vivo study, the hydrogel made of RADA16-SVVYGLR was compatible for cells proliferation in zebrafish embryos development. We expect that functionalized self-assembling peptide hydrogel would promote vessels outgrowth to regulate blood circulation in CNS, create a favorable microenvironment for neural tissue repair and serve as a potential therapeutic application in traumatic brain damages.

Session No.: Y07-04

Development of an Artificial Dermis Using Duck's Feet Extract Collagen

Ju Yeon Jeong[1], Jung Min Lee[1], Hyung Woo Ju[1], Bo Mi Moon[1], Hyun Jung Park[1], Ye Ri Park[1], Yeung Kyu Yeon[1], Md. Tipu Sultan[1], Vijay Kumar[1], Chan Hum Park[1]

[1]Hallym University

Collagen constituting the extracellular matrix has been widely used as biocompatible material for human use. In this study, we extracted collagen from duck’s feet by using a simple method without utilizing harsh chemical. We fabricated duck’s feet collagen scaffold for the artificial dermis.The duck’s feet collagen spongy sheet was prepared by freeze-drying. The resultant scaffolds were characterized for internal pore structure, surface hydrophobicity and other physicochemical characters, including mechanical properties by using different techniques such as Scanning Electron Microscopy (SEM) and Fourier Transform Infrared Spectroscopy (FT-IR). The cytotoxicity and cell infiltration studies were carried out after culturing NIH3T3 fibroblasts in presence of artificial dermis. We also investigated its regenerative effect on skin defects (1x1cm) in rats. One side was fixed with porcine collagen (Thera form®), and the other side was duck’s feet collagen spongy fixation.The histological examination using Hematoxylin and Eosin (H&E) and Masson Trichrome (MT) staining after (3, 5, 7, 14 and 21days), revealed that these collagen spongy can moderately be converted to artificial dermis compared to Thera form® . The results from gross findings showed that artificial dermis results in low contraction and less scar formation than the commercially available Thera form®. In this study, we suggested that duck’s feet collagen scaffolds could be used as the dermal substitute.

Session No.: Y07-05

Human Muscle-stuffed Vein Supports the Quality and Maturation of Myelinated Nerve

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The efficacy of the nerve conduits available in the market for the repair of peripheral nerve injury (PNI) is questionable. In this study, we investigated the efficiency of living biological conduit ie. cell-seeded muscle-stuffed vein (MSV) in the repair of PNI in terms of the quality and maturity of the myelinated axons.

Muscles, veins and bone marrow were obtained from donor with informed consent. One million neural differentiated human mesenchymal stem cells were transfected with green fluorescent protein (GFP) prior to seeding in the decellularized MSV. Athymic rats were used as the PNI model. Fifteen millimeters of sciatic nerve defect was created and bridged with rat autograft (RA), human cell-seeded MSV (SMSV) and unseeded MSV (MSV) (n=3). The quality and maturation of nerve regeneration were evaluated by assessing the morphological characteristics and expression of S100B, a marker for functional Schwann cells. Transmission electron microscopic and immunohistochemical analyses were performed.

Maturity of the newly regenerated was evident by the expression of S100B was present in all groups. Seeded-cells in SMSV group continued to survive and successfully grafted in the newly regenerated nerve evident by the presence of GFP. G-ratio (inner diameter/outer diameter of myelinated axons) in all experimental groups fell within the normal range of normal sciatic nerves (0.44–0.79). Interestingly, G-ratio and myelin thickness in SMSV group were significantly higher than that in MSV group ($p<0.05$) and no different from that in RA group. Axon diameters in SMSV group were larger than that in MSV group but smaller than that in RA group.

We demonstrated that muscle-stuffed vein can support nerve regeneration with mature myelinated axons in the repair of PNI. Quality of the newly regenerated myelinated axons was improved with the addition of neural differentiated mesenchymal stem cells. Further study will correlate these findings with additional functional outcome measures.

Session No.: Y07-06

Treatment of Pelvic Organ Prolapsed by Tissue Engineered Silk Fibroin Microsphere Incorporated with Adipose Derived Stem Cell Through Reversing the Extracellular Matrix Environment

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Pelvic organ prolapse (POP) is a common disease in the middle and old aged women. There is still a lack of etiological treatment for this disease. Our previous results showed that there was a disordered arrangement of the elastic fibers and a decreased expression of the elastin gene in the pathological sites of vaginal tissue compared with the normal sites from POP patients, this implied that the destruction of the pelvic floor microstructure may be the pathological factors of POP, which was also found confirmed by the loxl1 (loxl1 plays an important role in elastin synthesis and cross linking) knockout mice with a phenotype of rectal prolapse, and their synthesis and arrangement of elastic and collagen fibers were also disordered in their pelvic floor. This study aims to regulate the behaviors of stem cells to increase extracellular matrix synthesis through tissue engineering technology. The loxl1 gene knockout mice were used as an animal model. The silk fibroin microspheres were fabricated by incorporating with ADSCs (adipose derived stem cells) treated with TGFb1. The results showed that TGFb1 could increase the expression of LOX family members and elastin from ADSCs cultured in silk fibroin microspheres. In the next stage, the tissue engineered silk fibroin microspheres could be injected into the pelvic floor of model mice to increase the synthesis and cross-linking of the elastin, then to reverse the symptom of prolapse. This study would provide new ideas for the clinical treatment of POP.

Session No.: Y07-07

Fabrication of Pancreatic β-cell Spheroids in Polydimethylsiloxane Device in Different Oxygen Conditions

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One of the promising strategies to cure Diabetes Mellitus type I is islet transplantation. However, donor shortage makes it difficult to obtain sufficient amounts of islets, and long-term immunosuppressive therapy reduces quality of life. Therefore, engineering bio-artificial islets using pluripotent stem cells can be a potential solution. In this study, we fabricated a device which has ~1000 wells of 500-μm in diameter for culturing pancreatic β-cell spheroids. The device was made from oxygen permeable polydimethylsiloxane, facilitating the supply of oxygen to spheroids from the top and bottom through culture medium and polydimethylsiloxane. Using this device, we were able to grow spheroids of 200-300 μm in diameter uniformly. Immunohistochemical staining of spheroids showed that pancreatic β-cells, MIN6-m9, secreted more insulin than MIN6, and both spheroids secreted glucagon. Judging from SEM images, spheroids of both cell types appear to be round-shaped, but MIN6-m9 spheroids were partially fractured that indicates lower mechanical strength. Real-time RT-PCR analysis showed that after spheroids reached the stable size, INS1 and INS2 expressions increased comparing to monolayer culture. The upregulation was ~1.5 time for MIN6 and ~2.5 times for MIN6-m9. Comparisons of polydimethylsiloxane device with polymethylmethacrylate device (with the same design, but without oxygen supply through the bottom) revealed that oxygen supply played an important role in growth of spheroids and insulin secretion for both MIN6 and MIN6-m9. In future this methodic can allow fabricating pancreatic tissue with high insulin secretion rate. With combination of using pluripotent stem cells, this can become a way to overcome drawbacks of previous treatments of Diabetes type I and help to cure this disease.

Session No.: Y07-08

Sugar Fix for Skin Wound Healing

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One of the major growth factors crucial for wound healing is platelet-derived growth factor (PDGF), a family of heparin-binding proteins made by many cell types. The PDGF-BB variant is the only growth factor approved by the Food and Drug Administration for the treatment of non-healing diabetic ulcers. However, excessive usage of PDGF-BB has been linked to an increased risk of cancer. To eliminate the use of exogenous PDGF-BB, we isolated a variant of heparan sulfate (HS) targeting PDGF-BB that sequesters endogenous PDGF-BB. The addition of PDGF-BB binding HS protects PDGF-BB from degradation, resulting in an enhanced signaling and bioactivity in human dermal fibroblasts. Preliminary results show that PDGF-BB binding HS promotes revascularization and granulation tissue formation in a pig skin wound model. These results suggest that PDGF-BB binding HS accelerates skin wound healing by potentiating the bioactivity of PDGF-BB.

Session No.: Y07-09

Exploring Mussel Adhesive Protein-based Aligned Nanofiber Scaffold for Nerve Regeneration

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Among many fields of tissue engineering studies, neuronal regeneration is one of those big parts yet to be understood. In this study, we enhanced neural tissue regeneration by imposing engineering perspectives to mussel adhesive protein (MAP)-based scaffold. Specifically, a potential of recombinant MAP, which is a biomimicry of adhesive proteins from mussel byssus, was exploited as a promising biomaterial for the neural tissue regeneration. As a biomaterial, recombinant MAP was manipulated to endow the biomolecular aspects and the topological aspects. For the biomolecular aspects, peptide-conjugation was performed to forge the recombinant MAP into a nerve regeneration material. For the topological aspects, aligned nanofibrous structure of MAP was made to generate directional guidance, which is the key factor for the nerve regeneration. Consequently, MAP-based nerve conduit with aligned nanofiber structure was proposed to achieve advances in regeneration of a damaged nerve tissue.

Session No.: Y07-10

Parallel Topography Induced Cell Elongation Contributes to the Transdifferentiation of Dermal Fibroblasts to Tenocytes in Vitro and Neo-tendon Formation in Vivo

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This study aimed to examine the effect of aligned topography on cell elongation as well as its effect on tansdifferentiation of human dermal fibroblasts (hDFs) into tenocytes in vitro and form neotendon in vivo. In vitro, hDFs grown on a parallel microgrooved silicon membrane were induced into an elongated shape, and exhibited a predominant tenogenic phenotype via significantly enhanced expression of scleraxis, tenomodulin, collagens I, III, VI and decorin，but not the markers of other mesenchymal lineage. Cell elongation also promoted TGF-beta1, but not alpha-SMA expression. Additionally, elongating chondrocytes failed to induce a tenogenic phenotype. It was also observed that exogenous TGF-beta1 could significantly enhance cell-shape mediated tenogenic transdifferentiation, whereas TGF-beta neutralizing antibody, TGF-beta receptor signaling blocker, Rock inhibitor and cytochalasin D could block the transdifferentiation, indicating that that the synergistic effect between TGF-beta1 and cytoskeletal signaling plays a significant role in cell-elongation mediated tenogenic transdifferentiation. In vivo, after implantation of nanofibers seeded with hDFs for 3 months in nude mice, cells on aligned nanofibers formed much organized tissue structure along with stronger mechanical properties and higher expression levels of tenogenic markers when compared with random nanofiber group. Moreover, after implantation of cell-free nanofiber in situ to repair rat Achilles tendon defect for 3 months, the aligned nanofibers could better recruit host cells into the scaffold and regenerate a tendon-like tissue along with enhanced gene expression of tenogenic markers than the random nanofibers. Furthermore, the developed approach was also applied to tendon regeneration in rabbit Achilles tendon repair model, which showed that fiber alignment plus unilateral mechanical loading significantly enhanced in situ tendon regeneration. This study proved that basic concept that the topography of parallel alignment could induce transdifferentiation of fibroblasts into tenocytes, and this concept was further applied to in vivo tendon regeneration, and potentially for clinical trial.

Session No.: Y08-01

Enhanced Osteogenic Commitment of Human Mesenchymal Stem Cells on Polyethylene Glycol Cryogel with Graphene Oxide Substrate

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Tissue engineering is an interdisciplinary field that has attempted to restore or regenerate tissues and organs by biomimetic fabrication of scaffolds with functionality. Polyethylene glycol (PEG)-based cryogels are attractive biomaterials for stem cell culture due to their tunable material properties and porous structures. Graphene oxide (GO) is considered as a biomaterial with enormous potential due to its nontoxicity, high dispersity, and enhanced interaction with biomolecules. These characteristic of Graphene oxide (GO) can stimulate the interaction between substrate and cells. In this study, we utilized GO substrates to observe effects on the osteogenic responses of human tonsil derived mesenchymal stem cells. Compared to unmodified hydrogel, Graphene oxide (GO) embedded polyethylene glycol cryogel showed not only improved cell attachment, focal adhesion, and focal adhesion kinase (FAK) signaling activation, but also enhanced cell viability and survival. As a result, we demonstrated that PEG-GO cryogel can stimulate osteogenic differentiation under osteo-inductive condition and can enhance osteogenic phenotypes compared to the PEG cryogel group. All these data suggested that, GO could serve as an effective bio-functionalizing agent for hydrogels to control stem cell behaviors and lineage commitment.

Session No.: Y08-02

Proliferation and Osteogenic Differentiation of Human Amniotic Fluid Derived Stem Cells Cultured on Biomaterials Having Nanosegments and Optimal Elasticity

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Human amniotic fluid-derived stem cells (hAFSCs) are pluripotent fetal cells capable of differentiating into multiple lineages, including representatives of the three embryonic germ layers. AFSCs may become a more suitable source of stem cells in regenerative medicine and tissue engineering. However, stem cell characteristics, such as proper differentiation and maintenance of pluripotency, are regulated not only by the stem cells themselves but also by their microenvironment. Furthermore, physical characteristics of cell culture substrates such as substrate elasticity may influence the fate of stem cell differentiation. In this study, I investigated efficiency of osteogenic differentiation of hAFSCs cultured on cell culture substrates that have different elasticities and are immobilized with extracellular matrix-derived oligopeptides. The prepared dishes coated with polyvinylalcohol-co-itaconic acid (PVA-IA) films having different elasticities were prepared by controlling the crosslinking time in crosslinking solution that contains glutaraldehyde. The PVA-IA dishes were grafted with ECM-derived oligopeptides through N-(3-dimethylaminopropyl)-N’-ethylcarbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) chemistry in an aqueous solution. Five oligopeptides were selected to be grafted on PVA-IA hydrogels in our experiment. Pluripotent gene expressions (Nanog, Oct4 and Sox2) were evaluated by the qRT-PCR measurements. It is found that there is an optimal elasticity of cell culture matrix to keep pluripotency of AFSCs for their culture. To characterize osteogenic differentiation of hAFSCs, Alkaline phosphatase (ALP) activity, alizarin Red S staining and von Kossa staining of the cells on the dishes were evaluated after two weeks and four weeks of culture in induction media. It is suggested that physical cues such as stiffness of culture materials as well as biological cues of extracellular matrix components can guide and decide differentiation of hAFSCs into osteoblasts.

Session No.: Y08-03

Novel Cell-inspired Nanotherapeutics for Cartilage Regeneration

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Mesenchymal stem cell (MSC) therapy has been evaluated in both clinical and animal studies for treatment of cartilage injuries. As with all cellular therapies, there are logistical and operational challenges in maintaining cell vitality and viability. Increasingly, the efficacy of many MSC therapies has been attributed to secreted paracrine factors. Notably, exosomes are nano-sized (50-100nm), cell-secreted bi-lipid membrane vesicles that have been recently identified as the principal mediator underlying the biological effects of MSCs in immunomodulation and tissue regeneration. Here, we investigate the utility of MSC exosomes as a novel cell-free therapeutic to promote cartilage regeneration in a critical-sized osteochondral defect model in adult immunocompetent rats. Exosomes purified from conditioned medium of human embryonic MSCs were administrated intra-articularly after surgery and subsequently on a weekly basis over a 12-week period. Our results showed that intra-articular injection of MSC exosomes greatly facilitated orderly cartilage and subchondral bone regeneration. Macroscopically, defects treated with MSC exosomes showed improved International Cartilage Repair Society (ICRS) macroscopic scores than the contralateral saline-treated defects at 6 and 12 weeks. Histologically, by the end of 12 weeks, defects treated with MSC exosomes displayed a smooth continuous hyaline neocartilage layer rich in type II collagen and glycosaminoglycan, and a fully regenerated subchondral bone. In contrast, saline-treated defects showed poor surface regularity and filled mostly with fibrous tissues. Modified O'Driscoll scorings further indicated significant improvement in cartilage repair by exosome treatment compared with the contralateral controls at both 6 and 12 weeks ($P < 0.01$). Importantly, no adverse tissue reaction was observed in all animals. Taken together, this study provided strong evidence that human MSC exosomes promote orderly cartilage regeneration. Human MSC exosomes are promising safe, ready-to-use and 'cell-free' therapeutic for treatment of cartilage injuries.

Session No.: Y08-04

Using Baculovirus-Mediated miR-214 Sponges Switch Osteoporotic ASCs from Adipogenesis to Osteogenesis for Osteoporotic Bone Defects Repair

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Clinically, an imbalance between adipogenesis and osteogenesis in mesenchymal stem cells (MSCs) is observed in osteoporotic patients. Recently, many studies revealed aberrant MicroRNAs (miRNAs) expression in elderly osteoporotic patients, but the role of miRNAs between adipogenesis and osteogenesis draws much less attention. Here we attempted to switch osteoporotic adipose-derived mesenchymal stem cells (ASCs) from adipogenesis to osteogenesis for repairing osteoporotic bone defects through miRNAs regulation. We found that ASCs harvested from rats with long-term estrogen deficiencies exhibited over-expression of miR-214. Transduction of the osteoporotic ASCs with the baculovirus (BV) that exploited Cre/LoxP-mediated recombination for prolonged expression of miR-214 sponges persistently down-regulated the miR-214 expression. The miR-214 suppression switched the osteoporotic ASCs differentiation from adipogenesis to osteogenesis through Wnt signaling, as well as promoted the osteoporotic BMSCs osteogenesis via BMP7 in a paracrine fashion. To augment the in vivo bone healing, we co-transduced the osteoporotic ASCs with the hybrid BV expressing miR-214 sponge and another BV expressing BMP2. Allotransplantation of the BMP2/miR-214 sponges-expressing osteoporotic ASCs into the critical-size defect (3 mm in diameter) at the femur metaphysis of ovariectomised rat potentiated the bone healing and remodeling, filling 22.4% of bone volume/total volume (BV/TV) at 5 weeks. The BMP2/miR-214 sponges-expressing osteoporotic ASCs not only accelerated the healing, but also ameliorated the bone quality (density, trabecular number, trabecular thickness and trabecular space), as evaluated by micro computed tomography, histology and immunohistochemical staining. Altogether, this study paved a new avenue to treatment of osteoporotic bone defects using miRNA-modulated ASCs.

Session No.: Y08-05

Fabrication of Bioaborbable Bone Fixation System Using Silk Fibroin by Centrifugal Casting

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Bioresorbable fixation systems(PLGA, PLLA etc) have been popular for the treatment of bone fractures. However, their mechanical properties are not appropriate and its price is very expensive. To overcome these problems, we fabricated a bioabsorbable silk fibroin fixation plate/screw by using cetrifugal force casting method. The resultant plate/screw had been characterized for internal pore structure, surface hydrophobicity including mechanical properties, SEM and FTIR. Silk fibroin plate/screw were degraded within 49d in protease XIV in PBS. We investigated its regenerative effect on femur defects in rats. The silk fibroin plate/screw had a compressive pressure similar to that of a polylactic acid plate, and a tight, pore-free microstructure. Bilateral segmental bone defects (2-mm length) were created in the fumer of 12 adult rats. One side was fixed with the silk fibroin plate/screw, and the other side was bioresorbable plate/screw(BiosorbTM) fixation. Gross inspection revealed no specific complication. At 8 weeks postoperatively, the femur were explored by micro-computed tomography and histological examination. New bone formation and osteoblast activity were observed in sides treated with the silk fibroin plate/screw, and bony defects were completely healed within 8 weeks. These results suggest that the silk fibroin plate/screw is a potential candidate for a new bioresorbable fixation system.

Session No.: Y08-06

Physicochemical Characterization of a Novel Bioactive Ion-doped Calcium Silicate-based Injectable Bone Cement

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Injectable bone cements (IBCs) are an ideal form of bone graft substitutes, as it allows for minimally invasive procedures and fills complex-shaped defects. Current clinically available IBCs such as polymethylmethacrylate (PMMA) and calcium phosphate cement (CPC) possess several limitations which prevent them from being widely accepted among clinicians. We have previously developed a bioactive ion-doped calcium silicate strontium-doped hardystonite (Sr-Ca2ZnSi2O7, SrHT) ceramic which has been previously shown to possess excellent in vivo bioactivity. In this study, a novel injectable SrHT-based cement (SC) was developed to address the need for a bioactive, mechanically competent cement with good handling and injection properties. SC pastes showed very little exothermicity (100μm). Compressive strength of SC was 7.4±0.8MPa, 11.7±1.8MPa, 16.0±0.9MPa and 8.8±2.3MPa (±SD) after being immersed in 37oC deionized water for 2h, 1d, 3d and 7d respectively. The pH profile of simulated body fluid (SBF) with immersed SC remained slightly higher than physiological levels at all measured time points up to 24 days. SC discs showed extensive surface apatite formation when immersed in 37oC SBF after 1d. SC showed significantly higher radiopacity (3640±290 HU) compared to a cancellous bone model (640±160 HU) when observed under micro-computed tomography. SC demonstrated no toxic effect on primary human bone-derived cells. SC is a novel cement formulation that possesses a unique set of advantageous properties compared to those of PMMA and CPC. SC could be a promising IBC that can facilitate bone repair in vivo.

Session No.: Y08-07

Development, Evaluation and Mechanical Stimulation of a Tissue Engineered Three Dimensional Bone Cancer Metastases Model

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2D monolayer models are frequently used in drug screening for cancer studies. These in vitro 2D models do not represent the native physiological environments or cellular responses as well as the in vivo models. Current 3D in vitro models mimic only specific physiological responses in in vivo models and fail to consider the effects of mechanical loading. In this study, we employ tissue engineering technique for developing an in vitro, metastatic prostate cancer model of RFP transfected-prostate cancer cells and mesenchymal stem cells co-cultures. This was followed by application of cyclic mechanical loading on the 3D cancer models and analysis of associated effect on cellular proliferation. Cancer cell proliferation in the co-cultures on both 2D and 3D substrates were studied and characterized using fluorescence microscopy and qRT-PCR. Results showed that cancer cell proliferation rates in 3D models are lower than the 2D models. Cyclic compressive strains (2200μℇ, 1 Hz) on 3D models led to a decrease in RFP gene expression, which could indirectly indicate a reduction in the cancer cell numbers. To increase the physiological relevance of this model, 3D tumour spheroids were considered in the design of 3D metastatic cancer models. Process parameters to fabricate PC-3 multicellular tumour spheroids were optimized and characterized. Quantification of spheroid growths using gene expression means and image-based diameter measurements were found to be statistically equivalent to each other. Results suggest that image-based analysis could replace qRT-PCR quantification methods as a form of non-destructive method to measure tumour growth which would allow cost effective characterization, samples and resources could be saved. Proliferation rate of cancer cells in 3D spheroids were observed to be greater than cancer cells in 3D co-cultures. In conclusion, cyclic mechanical loading reduced cancer cell numbers. Future studies will analyse the effect of mechanical loading on tumour spheroids seeded models.

Session No.: Y08-08

The Synthesis of Porous Biodegradable Poly (Glycerol Sebacate) Scaffold with Hydroxyapatite as Bone Substitute

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Bone grafting is a common treatment for bone fracture and tumor by facilitating the regeneration of bone tissue. Autologous, allologous or synthetic grafts are often used in bone grafting, and implantation of synthetic scaffold causes less rejection and inflammation compared to ordinary bone graft. In this project, synthesis of biodegradable porous scaffold for orthopedic implant was developed. Poly(glycerol sebacate) (PGS) porous structure was manufactured by salt-leaching method, and hydroxyapatite (HAP) was added to increase mechanical strength and osteoconduction. Effects of different pore sizes and HAP ratio were studied. Porosity test showed that porosity remained 75 to 85% between different batches. Enzymatic degradation test showed that polymer erosion rate was effect by total surface area and amount of HAP. The compression strength ranged between 0.015 and 0.205 MPa. Preliminary rat calvarial defect model indicated minimum inflammation and superior recovery in 8 weeks. HAP-PGS porous scaffold was successfully fabricated, and biodegradability and mechanical strength could be modified for medical applications by changing the pore size and amount of HAP. From these results, utilization of PGS porous scaffold in clinical practice is promising.

Session No.: Y08-09

Fabrication of 3D Porous SF/β-TCP Hybrid Scaffolds for Bone Tissue Reconstruction

Hyun Jung Park[1], Hyung Woo Ju[1], Bo Mi Moon[1], Jung Min Lee[1], Ye Ri Park[1], Ju Yeon Jeong[1], Md. Tipu Sultan[1], Vijay Kumar[1], Chan Hum Park[1]

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Bio-ceramic is a biomaterial actively studied in the field of bone tissue engineering. β-tricalcium phosphate has been widely used in bone tissue engineering. But, only certain ceramic materials can resolve the corrosion problem and possess the biological affinity of conventional metal biomaterials. Therefore, the recent development of composites of hybrid composites and polymers has been widely studied. In this study, we aimed to select the best scaffold of silk fibroin and β-TCP hybrid for bone tissue engineering. We fabricated three groups of scaffold with SF, GS and GM, and we compared the characteristics of each group. During characterization of the scaffold, we used scanning electron microscopy (SEM) and a Fourier transform infrared spectroscopy (FTIR) for structural analysis. We compared the physiological properties of the scaffold to swelling ratio, water uptake and porosity. To evaluate the mechanical properties, we examined the compressive strength of the scaffold. During in vitro testing, we evaluated cell attachment and cell proliferation (CCK-8). Finally, we confirmed in vivo new bone regeneration from the implanted scaffolds using histological staining and micro-CT. From these evaluations, the fabricated scaffold demonstrated high porosity with good inter-pore connectivity, showed good biocompatibility and high compressive strength and modulus. In particular, the present study indicates that the GM scaffold using β-TCP accelerates new bone regeneration of implanted scaffolds. Accordingly, our scaffold is expected to act a useful application in the field of bone tissue engineering.

Session No.: Y08-11

Biomimetically Ornamented Rapid Prototyping Fabrication of an Apatite-Collagen-Polycaprolactone Composite Construct with Nano-Micro-Macro Hierarchical Structure for Large Bone Defect Treatment

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Objective: To construct a scaffold that matches function with structure, the combination of rapid prototyping (RP) technology and biomimetic functionalization fabrication strategy to construct a hierarchical composite scaffold consisting of natural and synthetic polymers as well as biomimetic ceramics holds great promise. Methods: Macro-porous PCL framework was fabricated using RP technology, then it was meticulously functionalized through collagen evacuation and simulated body fluid biomimetic deposition. The biomimetically functionalized scaffold (apatite−collagen−polycaprolactone, Ap-Col-PCL) was characterized by SEM, XRD, FTIR and mechanical compression test. Moreover, the biocompatibility and osteoinductivty of Ap-Col-PCL scaffold was evaluated by in vitro cell culture assay and in vivo critical-sized bone defect implantation in rabbit radius. Results: Ap-Col-PCL scaffold was characterized with hierarchical architectures of a nanoscale (∼100 nm thickness and ∼1 μm length) platelike apatite coating on the microporous (126 ± 18 μm) collagen networks, which homogeneously filled the macroporous (∼1000 μm) PCL frameworks and possessed a favorable hydrophilic property and compressive modulus (68.75 ±3.39 MPa) similar to that of cancellous bone. Moreover, in vitro cell culture assay and in vivo critical-sized bone defect implantation demonstrated that the Ap-Col-PCL construct could not only significantly increase the cell adhesion capability (2.0-fold) and promote faster cell proliferation, but also successfully bridge the segmental long bone defect within 12 weeks with much more bone regeneration (5.2-fold), better osteointegration (7.2-fold), and a faster new bone deposition rate (2.9-fold). Conclusions: Our study demonstrated that biomimetically ornamented Ap-Col-PCL scaffold exhibits a favorable mechanical property, more bone tissue ingrowth, and better osteointegration capability as an effective bone graft substitute for critical-sized bone defect treatment; meanwhile, it can harness the advantages of RP technology, in particular, facilitating the customization of the shape and size of implants according to medical images during clinical application.

Session No.: Y09-01

Design of Microfluidic Devices for Vasculature Regeneration Using Murray's Law

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As the number of patients in the waiting list for organ transplants increases every year, organ repair and regeneration has rapidly become one of the solutions to supply the overwhelming demand of compatible organs. In response to the lack of organ donors and the severe immune reactions post-transplant, it is believed that tissue engineering will provide a better alternative. However, solid units with a complex vascular network are harder to grow artificially due to the lack of blood vessels to supply the nutrients they need, causing them to die and making the developed tissue worthless. The fabrication of microfluidic devices aims to mimic the complex microvascular system of organs such as kidney and liver to provide the sufficient oxygen for proper organ regeneration. Existing bifurcated network in microfluidic systems were designed based on constant cross section of parallel channels. However, by basing the design instead on constant shear stress across parallel channels, the microvasculature regenerated is more likely to be mechanically similar to natural vasculature according to Murray’s Law. Through computer-aided design and laser ablation fabrication, this work presents new microfluidic devices with improved hydraulic resistance. Endothelial cells were seeded in the devices for comparison between traditional and new designs. The ultimate goal is to develop a fully functional 3D synthetic microvascular structure that provides endothelial cells with a suitable environment for proper proliferation and cell function.

Session No.: Y09-02

Baculovirus-Engineered Adipose Stem Cell Sheet Persistently Expressing GDNF Enhance Sciatic Nerve Regeneration

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Peripheral nerve regeneration is dependent on the efficacy of axonal outgrowth from the survival of axotomized neurons. It may take up to six months for complete recovery from losing motor or sensory function, but more than half of patients had poor results. Stem cell gene therapy provides the opportunity to overcome the problem for these patients. Here we constructed a hybrid baculovirus vector that persistently expressed glial cell line-derived neurotrophic factor (GDNF) that plays diverse functions during the nerve repair. After transduction, the recombinant baculovirus enabled the transduced adipose-derived stem cell (ASC) sheet to express GDNF for >20 days and recruited Schwann cells in vitro. In vivo, rat sciatic nerve were transected and wrapped by GDNF-expressing cell sheet. Gait analysis, conduction velocity and nerve impulses was carried out at 8 weeks and improved nerve regeneration was observed in the transplant group compare to the microsurgical repair alone. Moreover, the Luxol fast blue stain result demonstrated a significant increase in the number of myelination axon in the distal nerve stump. Our data show that combination of ASC cell sheet and baculovirus-mediated GDNF expression exhibited a synergistic effect in promoting nerve regeneration, and could provide an alternative stem cell gene therapy for nerve repair.

Session No.: Y09-03

Silk Fibroin Induces Cell Proliferation and Wound Remodeling in Wound Healing Process by NF-kB Signaling Pathway not Mapk Signaling

Ye Ri Park[1], Hyun Jung Park[1], Bo Mi Moon[1], Hyung Woo Ju[1], Jung Min Lee[1], Yeung Kyu Yeon[1], Md Tipu Sultan[1], Vijay Kumar[1], Chan Hum Park[1]

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Wound healing is a comprehensive and complex mechanism, which is dependent upon many interactions between cells and molecules. In normal skin, fibroblasts are responsible for the regeneration of connective tissue and tissue remodeling. The wound healing process and NF-κB signaling pathway share a close connection at the molecular level. Cyclin D1 is a NF-κB target gene and is also associated with wound healing and cell proliferation. Silk fibroin (SF) is a natural protein derived by Bombyx mori. The use of silk in tissue engineering and as a wound dressing has several advantages such as highly biocompatibility, low immunogenicity, minimal toxicity, non-carcinogenicity and an adjustable degradation rate. For this reason, the application of SF in these fields has been widely reported. To name a few, SF has been used to produce artificial bone, skin, esophagus, cornea, and wound dressings. In previous studies, SF has shown remarkable tissue regenerative activity in many cell types. However, the signaling pathways remains unknown. Thus, understanding the signaling pathways responsible for the tissue regenerative effects of SF would lead researchers towards the production of more effective mixtures and would be a major advance in the fields of tissue engineering and medicine.

Session No.: Y09-04

Effects of Physical Loading and Melatonin on 3T3-L1 Preadipocytes

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Physical loading is an important regulator of fat tissue. Melatonin is a hormone secreted by the pineal gland that is involved in the regulation of body fat and weight. Differentiation of preadipocytes leads to an increase in mass of adipose tissue. In this study, we investigated the effects of melatonin and fluid shear stress in adipose tissue maintenance via control of cell death and differentiation using preadipocytes. Preadipocytes were cultured in Dulbecco's modified eagle's medium (DMEM) using 5% calf serum at 37℃ in a humidified 5% CO_2 incubator. For physical loading, preadipocytes were stimulated with a maximum dynamic fluid shear stress of 1 Pa at 1 Hz for 1 and 2 hours with/without melatonin (100 nM). Nuclear morphology was observed by 4', 6-diamidino-2-phenylindole (DAPI) staining. ERK, p-ERK, and PPAR gamma proteins were assessed by Western blot analysis. GAPDH was used as a house keeping gene. Activated ERK regulates cellular activities such as cell growth and differentiation while PPAR gamma is one of the key regulators of adipogenesis. Expressions of p-ERK and PPAR gamma increased as the duration of fluid shear stress increased. Fluid shear stress (2 hr) and melatonin (100 nM) in combination increased p-ERK expression compared with control. However, fluid shear stress (1 hr) with/without melatonin (100 nM) decreased p-ERK expression compared with control. These preliminary results indicate that fluid shear stress may promote the differentiation and adipogenesis of preadipocytes especially in longer duration stimulation. These results may be important for maintenance or regeneration of adipose tissue. Furthermore, we expect to find significant relationships between melatonin and adipose mechanobiology.

Session No.: Y09-05

Transforming Growth Factor-β Inhibits Adipogenesis in Regenerating Glycerol-injured Muscle

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Introduction: Fibrosis and adipogenesis are characteristic features of several muscle diseases such as muscle dystrophies, inflammatory myopathies and sarcopenia, they negatively affect muscle function. Chemically-induced injuries provide good models to study the mechanism of muscular dystrophies and consequently to develop new therapies for treatment. Recently we have reported that glycerol injury induced muscle regeneration with adipogenesis and progressive deposition of intramuscular connective tissue in normal mice [1]. However, the effect of transforming growth factor-β (TGF-β) on skeletal muscle regeneration and adipogenesis is unclear. Aim: The aim of the present study is to investigate the effect of TGF-β on muscle regeneration and adipogenesis following glycerol injury. Methods: Mice were divided into three groups. The early treatment group was injected with TGF-β combined with glycerol. The late treatment group was injected with TGF-β at day 4 after glycerol injury. The control group was injected with glycerol only. Injections were performed into tibialis anterior muscles of adult mice. Muscle samples were collected at day 7 after glycerol injury. Results: Early TGF-β treatment inhibited adipogenesis significantly while late treatment decreased adipogenesis. Moreover, muscle regeneration was impaired in TGF-β treated muscles compared to the glycerol-injured muscles. Furthermore, TGF-β reduced macrophages infiltration resulting in significantly larger necrotic area compared to glycerol-injured muscle. On other hand, TGF-β injection reduced mRNA expression of both myogenic and adipogenic factors compared to the glycerol-injured muscle. Conclusion: The inhibitory effect of TGF-β is much higher during early stages of muscle regeneration and adipogenesis. Keywords: Adipogenesis, glycerol, muscle regeneration, TGF-β Bibliography: 1. Mahdy MAA; Lei HY; Wakamatsu J-I; Hosaka YZ, and Nishimura T. Comparative study of muscle regeneration following cardiotoxin and glycerol injury. Annals of Anatomy - Anatomischer Anzeiger 2015; 202, 18-27.

Session No.: Y09-06

Assembling Composite Dermal Papilla Spheres with Adipose-derived Stem Cells to Enhance Hair Follicle Induction

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Intradermal adipose and precise cell composition within hair follicles create proper microenvironment for promoting hair neogenesis. However, due to lacking knowledge for the regulation of these factors in hair regeneration, it remains an enormous challenge to reconstruct functional hair follicles in vitro. In our lab, we were able to culture DP cells in three dimensional structure by forming DP spheres in unite size and modulate sphere characteristics through involvement of adipose-derived stem cells. The study aims to create a hair follicle model optimized for hair regrowth through assembling dermal papilla (DP) cells and adipose-derived stem cells (ASCs) in different strategies. To preserve DP markers after isolation, we seeded DP on chitosan-coated surface to form spheres. Besides, ASCs were co-cultured with DP by different assembling approaches; a mixed sphere of ASCs with DP cells (MA-DPS), or a core-shell structure, outer ASCs shell and an inner DP core (CSA-DPS). Sphere formation significantly maintained the characteristic of DP. Administration of ASCs, especially in CSA-DPS assembling strategy, further promoted the expression of DP markers and functional alkaline phosphatase activity of the DP cells. However, differentiated adipocytes could not facilitate those cell function. The in vivo patch assay demonstrated that CSA-DPS developed into hair-like structures which could barely be found in MA-DPS treatment. Cell tracing technique and immunohistochemistry staining indicated that the core-shell structure of CSA-DPS showed the ability to trap ASCs and promoted PPAR-alpha signal in ASCs which then induced the greater hair induction than MA-DPS. Taken together, ASCs exhibited great potential to promote hair follicle function through PPAR-alpha pathway. Furthermore, core-shell structure provide a suitable microenvironment to facilitate cell interaction between DP and ASCs.

Session No.: Y09-07

Key Technologies Research on Optimization of Chondrogenesis Based on Proteomics Map and Spatio-temporal Analysis of Microfracture

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Key technologies research on optimization of chondrogenesis based on proteomics map and spatio-temporal analysis of microfracture Dan Zhao1, Zigang Ge1 * 1Department of Biomedical Engineering, College of Engineering, Peking University, Beijing, China Introduction: As the most widely used means in articular cartilage regeneration treatment, The current research focuses on the microfracture in long-term operation improvement, postoperative clinical effect evaluation and the use of biological materials to promote chondrogenesis, and the lack of microfracture surgery research on migration, accumulation and differentiation of stem cells and chondrocytes and lymphocytes, macrophages. Moreover, there is a lack of spatio-temporal analysis research on key differentiation factors and inflammatory factors. The lack of the basic data hinders the efforts to improve clinical efficacy of microfracture. Materials & Methods: We carried on microfracture operation for full thickness defect rat. At different time after the operation, we used flow cytometry instrument to determine the cell concentration and composition entering into the bone marrow cavity. We also adopted proteomic methods to determine the concentration of growth factor and inflammatory factors. Results & Discussion: The results showed that the concentration of macrophage maintained at a high level in the bone marrow cavity after microfracture, while the concentration of growth factors BMP2、 TGF-β3、 Wnt3a、 gremlin2、 Noggin begined to increased gradually decreased later, inflammatory factors IL-1， TNF-α， MMPs maintained at a high level all the time after microfracture operation. Conclusion：An important reason why mesenchymal stem cells differentiated into fibrocartilage not hyaline cartilage is that there are less growth factors but more inflammatory factors. Key words: microfracture, growth factor, inflammatory factors.

Session No.: Y09-08

Three-layered Scaffolds for Artificial Esophagus Using Poly (E-Caprolactone) Nanofibers and Silk Fibroin: An Experimental Study in a Rat Model

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The purpose of this study was to determine the feasibility of an artificial esophagus using a three-layered poly(e-caprolactone) (PCL)-silk fibroin (SF) scaffold in a rat model. The artificial esophagus was a three-layered, hybrid-type prosthesis composed of an outer and inner layer of PCL with a middle layer of SF. After depositing the inner layer of the PCL scaffold by electrospinning, the lyophilized middle SF layer was created. The outer layer of PCL was produced following the same procedure used to make the inner PCL layer. Eleven rats were anesthetized using inhaled anesthesia. Circumferential defects of the cervical esophagus (n=11) were created and reconstructed. Groups of rats were sacrificed after the 1st and 2nd weeks. Three rats died of an esophageal fistula and wound infection. No gross evidence of a fistula, perforation, abscess formation, seroma accumulation, or surrounding soft-tissue necrosis was observed in the other rats sacrificed after the 1st and 2nd weeks. The artificial esophagus constructs produced complete healing of the circumferential defects by the 2nd week. The composition of the three-layered artificial esophagus was confirmed histologically to have an outer and inner layer of PCL and a middle layer of SF. The fusion of the PCL-SF scaffold and the regenerative tissue remained intact. Our study proposes a more practical experimental model for studying a three-layered PCL-SF scaffold in the esophagus. However, further studies on circumferential defect reconstruction in a rat model are still required.

Session No.: Y09-09

Inhibition of Rac1 Activity by Controlled Release of NSC23766 from Chitosan/β-Glycerophosphate Thermosensitive Hydrogel Effectively Ameliorates Calcific Tendinopathy Development in Vivo

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Background Calcific tendinopathy is a degenerative disease characterised by fibro-chondrocyte formation and calcification. Recent studies showed that Rac1 promoted chondrocyte proliferation, differentiation and pathological calcification. These findings warrant further investigations on the roles of Rac1 in Calcific tendinopathy development and therapy in animal models. Objective To investigate the role and mechanistic pathway of Rac1 involvement in pathological changes of tendon stem/progenitor cells (TSPCs) in vitro and calcific tendinopathy development in vivo, as well as to develop a strategy of modulating Rac1 activity for calcific tendinopathy treatment. Material and methods Calcific endinopathy and normal cartilage from human or rats were used for immunohistochemical study and Rac1 activity assay. TSPCs treated with IL1β and the untreated control were subjected to the Rac1 activity assay. To evaluate the effect of Rac1 in calcific tendinopathy development, a tendinopathy model was created by injection type I collagenase in mice. NSC23766 was injected subcutaneously. Tendons were subjected to histological analysis. Results It was found that there is aberrant Rac1 activation in human calcific tendon. Rac1 activity could also be elevated by IL1β. Additionally, inhibition of Rac1 activity by NSC23766 could protect tendon phenotype, restrain the tendon destruction by regulating catabolic factors and calcification related factors, and suppress PBMCs proliferation in vitro. Therefore, we developed a strategy of controlled release of NSC23766 from chitosan/β-glycerophosphate thermosensitive hydrogel to tendinopathy tendon, which effectively protected tendon from calcification. Conclusions These findings demonstrated that Rac1 activity is implicated in calcific tendinopathy development. Also, controlled release of Rac1 inhibitor is a promising strategy for calcific tendinopathy treatment.

Session No.: Y09-10

The Effect of Fiber Size of Electrospun Scaffolds on Annulus Fibrous-derived Stem Cells

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Background: Application of micro-/nanofibers as scaffold is a promising approach for annulus fibrous (AF) tissue engineering. As the outer region of AF is rich in type I collagen and composed of larger fibers, while the inner region is rich in type II collagen and composed of smaller fibers, mimicking the size of collagen fibers may facilitate AF tissue reconstruction. In this study, we prepared fibrous poly(L-lactic-acid) (PLLA) scaffolds of different fiber sizes and studied their effect on the differentiation of annulus fibrous-derived stem cells (AFSCs). Methods: PLLA fibrous scaffolds were fabricated using electrospinning. After AFSCs were cultured on the scaffolds for 7 days, their morphology was checked and expression of Col-I, Col-II, Aggrecan genes was quantified by RT-qPCR and the related proteins were analyzed by ELISA. Results：PLLA fibrous scaffolds with fiber diameter ranged from 3 μm to 8 μm were fabricated. AFSCs were round on the scaffolds of small fiber diameter, while became spindled on the scaffolds of large fiber diameter. After 7 days, expression of collagen-II and aggrecan genes decreased with fiber diameter, whereas gene expression of collagen-I increased. The related proteins level was similar to gene expression. Discussion and Conclusion: The scaffolds of different fiber diameters may mimic the outer, middle, and inner fibrous structures of native AF tissue. The gene expression and protein production of AFSCs on scaffolds with different fiber diameters was similar to that of AF tissue. Therefore, this study provides solid basis for the use of biomimetic scaffolds which mimic different AF zones for AF tissue engineering.

Session No.: Y10-01

Extracellular Matrix Plays a Pivotal Role in Maintaining the Stemness or Directing Tenogenic Differentiation of Cultured Adipose Derived Stem Cells

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Extracellular matrix (ECM) is the key component of tissue microenvironment, which could direct mesenchymal stem cell (MSC) fate by its chemical signals of matrix molecules and contained growth factors and physical signals derived from the matrix topography. This study aimed to examine the effect of ECM derived from different cell types on the stemness and phenotype of cultured adipose derived stem cells (ASCs). Human bone marrow stem cells (BMSCs) were cultured on dishes to produce ECM and then decellurized to generate cell-free BMSC-ECM (kindly provided by Dr. Xiaodong Chen at University of Texas at San Antonio). Compared to culture dish control, mouse ASCs cultured on BMSC-ECM exhibited greater proliferation potential, colony forming efficiency, and greater differential potentials towards adipogenic, osteogenic and chondrogenic lineages. We then further investigated the effect of tissue specific ECM on ASC differentiation. Porcine tenocytes and dermal fibroblasts were respectively harvested and grown on culture dishes until abundant ECMs were achieved, and then decellularized to generate tendon-ECM and dermis-ECM coated dishes. After the culture of hASCs on two types of ECMs, ASCs on coated dishes exhibited greater proliferation potential than non-coated dishes, whereas dermal-ECM seemed to be relatively greater to tendon-ECM. Compared to the former, tendon-ECM could significantly upregulate the tenogenic gene expression of sceleraxis, tenomodulin, collagens VI, III, decorin, and tenasin-c. Additionally, tendon-ECM could significantly down-regulate the expression of adipogenic genes of C/EBP, PPAR-gamma and Adipnectin compared to dermal-ECM. In addition, AKP and OCN gene expressions were much higher in dermal-ECM than tendon-ECM, while chondrogenic marker expressions were similar between two groups. Furthermore, preliminary data showed that bovine tendon tissue derived ECM exhibited greater tenogenic differentiation induction potential of hASCs than bovine dermis ECM. These results indicate that ECM could serve as the key component to generate bioactive scaffold for various tissue regeneration including tendon.

Session No.: Y10-02

Biofabrication and 3D Printing in Regenerative Medicine

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The ability of bone to self-repair after fracture is limited according to the extent of the damage; small fractures are usually able to heal perfectly, but larger fractures, known as segmental bone defects (SBDs), can leave permanent damage. The common treatment for SBDs is an autologous bone graft, which involves harvesting of non-essential bone, for example the iliac crest. However, this method is limited by the availability of bone, donor site morbidity, risk of infection and geometric mismatch between the harvested bone and the defect site, which can result in voids and poor integration. Repairing SBDs remains a major surgical challenge and suboptimal outcomes can have significant socio-economic repercussions and negatively affect quality of life. Over the past 30 years, a wide range of innovative synthetic materials have been developed to overcome the problems associated with autologous bone grafts. These materials include bioceramics (typically calcium phosphates (CaPs) and bioactive glasses), polymers (naturally derived such as collagen-I and synthetic such as polycaprolactone (PCL)) and hybrid materials (a mixture of bioceramics and polymers). None of these materials have had the strength required to withstand static and cyclic loads in vivo whilst maintaining sufficiently high porosity to facilitate bone ingrowth, vascularisation and the transport of nutrients. This study focuses on demonstration of implementing additive manufacturing techniques on design of scaffolds to tailor their properties for regeneration of SBDs.

Session No.: Y10-03

Mechanically Tailorable Hydrogels to Improve Cell Viability in 3D Bioprinting

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One of the key aims of tissue engineering is to precisely organize cells within a hydrogel matrix that will direct their fate and function by mimicking the mechanical properties of the natural extracellular matrix. Additive manufacturing technique used for tissue engineering, so called bioprinting, provides the possibility to realize 3D structures of complex living tissues. Using this approach, micro-valve based 3D bioprinting allows for the rapid and precise dispensing of cell loaded hydrogel droplets. However, this technique requires to formulate hydrogel blends for each application and therefore to adapt the printing process, especially to limit shear and temperature stresses that can impact cell behavior and viability. Herein, we present novel carboxylated agarose with an extent of 28, 60, and 93 % carboxylation at the C-6 of the D-galactose of the repeat unit as printable and tailorable hydrogel matrices for 3D bioprinting. Comprehensive rheological and mechanical studies revealed that these materials span the full range of shear and elastic modulus of natural tissues while exhibiting the same viscosity under printing conditions. As a result, we demonstrate versatile micro-valve printing to manufacture 3D structures comprised of defined domains of stiff and soft hydrogels while showing limited cell death under identical printing conditions. These mechanically tailorable hydrogels pave the way towards 3D printing of complex architectures in which cells can be placed into hydrogels of different stiffness mimicking the organization of functional tissues.

Session No.: Y10-04

Biomimetic Stimulation of Cells Using Self-assembled Silk Topographies

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Nanoscale topographical cues modulate cellular adhesion and can impart effects on cellular functions including neuronal differentiation. Regulating cell–surface interactions at the bio-interface is needed for designing intricate, biocompatible nano-materials or nano-devices in bio-medicine. Proteins being the most abundant extra-cellular component are promising candidates for designing such cell stimulating nano-topographies. The biocompatibility, bio-degradability, eco-friendly aqueous processing, and hierarchical assembly of structural blocks within the native silk protein fibroin ease controllable regulation of its architecture at the nano-scale. Here, we present the bio-mimetic nano-topographical assembly of silk fibroin mediated by electrostatic interactions on precisely engineered aminated silica surfaces. The assembled nano-topography closely mimics the architecture of a natural cypress leaf, the structural analogue of the neuronal cortex. When interacting with neuron cells (PC12), the topographical surface facilitates cell anchorage and stimulates cytoskeletal extensions into neuron-like phenotypes even in the absence of neuronal growth factor stimulation. This indicates far reaching implications of this self-assembled topography in nano-biotechnology and nano-biomedicine (supported by Global Research Laboratory (GRL) Program (Grant no. 2015032163), Priority Research Centers Program (Grant no. 2009-0093829) and KRF R-2015-01277 through the National Research Foundation (NRF), Republic of Korea).

Session No.: Y10-05

Peptide-modified Silk Fibroin Hydrogels with a Vascular Inducible Function for Soft Tissue Regeneration

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In the field of soft tissue engineering, hydrogels have been focused on not only as fillers but as biodegradable scaffolds to enhance tissue regeneration. To achieve the mechanical matching, the strength of hydrogels needs to be adjustable without using toxic cross-linkers such as glutaraldehyde. Bombyx mori silk fibroin can be processed into physical gels, whose storage modulus is adjustable from several Pa to 150 kPa by fibroin concentration. However, cell infiltration and vascularization into fibroin gels cannot be expected because fibroin has no cell-adhesive sequences such as RGD. Here, we produced a peptide composed of a fibroin H-chain repeat peptide, an REDV peptide, a MMP-2 cleavage peptide, and a VEGF mimic peptide. This peptide was introduced into a fibroin gel to improve the cell infiltration and vascularization. As the peptide showed enhancement effects on HUVEC adhesion and proliferation in vitro, it was mixed with fibroin and citrate buffer to form a peptide-modified fibroin gel. The resultant gel was embedded subcutaneously in rats for eight weeks. H&E staining showed the peptide promoted cell infiltration and vessel formation in fibroin gels in vivo. Particularly, at eight weeks post-implantation, the percentage of vessel area relative to the total gel area in the peptide-modified fibroin gel was twice as much as that in a fibroin gel without the peptide modification. Immunostainings of CD31 (endothelial cell marker), CD68 (macrophage marker), and P4HB (fibroblast marker) revealed that endothelial cells infiltrated into the peptide-modified fibroin gel to lead a cell group including macrophages and fibroblasts from one to four weeks post-implantation. In contrast, the cell group was absent and collagen fibrils were observed in the peptide-modified fibroin gel at eight weeks. Therefore, the peptide-modified fibroin gel led not to chronic inflammation but to the regeneration of dermal tissue, showing its availability for soft tissue angiogenic therapy.

Session No.: Y10-06

Micropatterning of Living Cells by Direct Inkjet-printing into Liquid Medium

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Micropatterning strategies have been considered as an effective platform for modeling in vivo microenvironments by controlling over cell and tissue architecture. Micro-engineering techniques usually modify the chemical properties of cell culture substrates to restrict the location and shape of the substrate region for micropatterning of cells. The patterning methods include masking, molding, photolithography, and microfluidics. However, those conventional patterning methods require significant protocol optimization as well as precisely engineered culture surface for reliable and reproducible patterning, and have a limit to pattern different kinds of multiple cells in a single plate. Here, we present a simple, fast, and high-resolution inkjet printing method for the reorganization of mammalian cells in a standard cell culture medium-filled tissue culture plate. The piezo-type inkjet printer generates cell-laden picolitre droplets with the small number of cells, typically 2-3 cells per droplet, and deposit them directly into the medium-filled plate at a pre-defined position with high accuracy. The direct cell-printing technique does not involve any complicated fabrication step to precisely pattern the surface of the plate, but still have abilities to control shapes in a 100-um scale. The cell printing process and parameters have been systematically optimized for high accuracy and reproducibility of the patterns as well as high viability of the directly printed cells, including a stand-off distance between a nozzle and plate, velocity of a moving stage where the plate is placed, a drop firing frequency and volume of medium in the plate. The printed cell patterns include squares, circles, stripes, curved lines and the technique also allows us to place two or more multiple types of cells in a single plate. We believe that the inkjet-based cell printing is a simple and versatile method to offer opportunities to replicate the complex architecture, boundary conditions, and characteristic of in vivo tissues.

Session No.: Y10-07

Acoustic Assembly of Cells into 3D Microtissues for Bottom-up Tissue Engineering

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Introduction Bioengineering of in vitro 3D tissue model is motivated by broad applications in drug screening, tissue engineering, and regenerative medicine. Although several techniques have been demonstrated to fabricate tissues with desired cell population and spatial organization, few of these methods allow generation of tissues with high cell packing density such as liver, heart and brain, where cell proximity is crucial to maintain cell viability and function. Here, we demonstrate a unique acoustic assembly technique to rapidly bioengineer 3D microtissues with a predefined architecture and physiologically-relevant cell packing density by scaffold-free assembly of cells / cell spheroids and subsequent tissue encapsulation and cell self-organization. Methods A custom-made shallow liquid container was used as an acoustic resonator and mounted on top of the vertical vibration generator. Cells/cell spheroids suspended in the fibrin prepolymer were loaded into the liquid container with a brim full boundary condition. Faraday waves were excited by applying vertical vibration on the liquid container via the vibration generator. Results/Discussion We uniquely explore Faraday waves as a noninvasive acoustic field to bioengineer 3D microtissues in vitro with a high cell packing density (108-109 cells mL-1) and predefined structure. Faraday waves at the liquid surface create a velocity field inside the liquid layer, which further results in a hydrodynamic drag field on the substrate of the liquid container to drive initially randomly distributed cells to the nodal regions beneath the Faraday waves and form closely packed 3D structures. We demonstrate this technique by generating 3D tissues for diverse organs, including liver, heart and brain. Metabolic assays indicated cells in the generated tissues have high cell viability and growth rate. Immunofluorescence analysis indicated the bioengineered microtissues have physiologically-relevant tissue functions and structures. We expect this technique will find broad applications in bottom-up tissue engineering.

Session No.: Y10-08

The Degrading Ca-Si-Mg 3D-scaffolds to Improve Angiogenesis and Osteogenesis of Human Periodontal Ligament Cells

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The purpose of this study is to develop a suitable degrading Mg–calcium silicate scaffolds (Mg–CS) and investigate the scaffolds to stimulate human periodontal ligament cells (hPDLCs) behavior. We show that MG-CS scaffolds can be reproducibly manufactured with a scaffold morphology highly resembling that of the pure CS scaffolds. The results show that the degradation rate of Mg–CS scaffolds depends on the Mg content in CS. All scaffolds were shown to be non-cytotoxic, and supported cell adhesion, proliferation, and differentiation. In addition, the inclusion of CS promotes precipitation of apatite on the scaffold surfaces which leads to earlier hPDLCs differentiation and matrix mineralization. The research results also suggest that Mg–CS scaffolds with this modified composition stimulate hPDLCs behavior and so may be good biomaterials for bone substitutes and hard tissue regeneration applications as they stimulate osteogenesis/angiogenesis.

Session No.: Y10-09

Biomimicking Platelet-monocyte Interactions as a Novel Targeting Strategy for Acute Myocardial Infarction

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Development of effective cardioprotective treatment strategies continues to be a challenge as many potential cardioprotective drugs fail to translate from the bench into clinical results. One of the key issues is the optimization of targeting to the infarcted heart. Although several drug delivery systems claims to actively deliver encapsulated drugs to the infarct area, the functionalized surfaces on these delivery systems only allows them to be better retained at targeted sites or have a higher circulation half-life. Thus, an enhanced permeability and retention (EPR) effect is still required as a main route of delivery for these delivery systems. Herein, we present an alternative that allowed cardioprotective drugs to be actively delivered without relying on the EPR effect. To mimic platelet interaction with the circulating monocytes during post-myocardial infarction (MI), platelet-like proteoliposomes (PLPs) were fabricated using purified human platelet membrane proteins and 1,2-dioleoyl-sn-glycero-3-phosphocholine (DOPC) lipids. In vitro data showed that PLPs displayed a strong affinity for monocytes and macrophages but not for endothelial cells. Intravital multiphoton imaging revealed PLPs had better targeting to the tissue injury site than the plain liposome control. When injected at 72 hours of reperfusion, which is when the monocyte recruitment is at its maximum, there were significantly more PLPs compared to the plain liposomes in the infarct area of the heart. Moreover, cobalt protoporphyrin (CoPP) encapsulated in PLPs (PLP-CoPP) was shown to improve the cardiac function in a murine model of MI while reducing the adverse effect of the encapsulated drug.

Session No.: Y10-10

Tailoring Polydimethylsiloxane (PDMS) Substrate for Mesenchymal Stem Cell Based Cell Sheet Engineering

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Poly(dimethylsiloxane) (PDMS) carries numerous advantages over other materials due to their transparency, low cost, ease of fabrication, readily moldable into sub-micrometer designs and its tunable mechanical properties. Nevertheless, the surface compatibility of PDMS for cell culture is often discouraging due to their high surface hydrophobicity of PDMS, which limits the affinity of mammalian cells to effectively adhere to the native PDMS surface. Herein, surface modification of PDMS ranging coupled with plasma treatment and fibronectin have been investigated to render a biocompatible PDMS surfaces for long term application in cell culture. While PDMS is not a suitable scaffolding materials in tissue engineering, its tunable mechanical properties and readily moldable properties inspired us to modified the surface properties and microtopography that could be used to direct and enhance mesenchymal stem cells (MSCs) based cell sheet development for later multi-lineage differentiation. In our studies, plasma treatment coupled with extracellular matrix on PDMS substrate has been established to prolong cell culture. To extend the application of PDMS in tissue engineering application, the substratum properties of PDMS were varied by tuning its mechanical properties or incorporation of microtopographies to study their effect of mesenchymal stem cell based cell sheet engineering and thereby developing a scaffold free tissue for future tissue regeneration. It was shown that different substratum properties exhibited MSCs based cell sheet with different multipotency profile while incorporation of microtopographies influenced the cellular/matrix arrangement. Hereby, we have established various surface modification approaches to enhance and stabilize long term cell culture and MSCs based cell sheet integrity and multipotency, which had significant implications for future scaffold free MSCs-mediated tissue regeneration.

Session No.: Y11-01

A Novel Strategy Including Immune Cells for Evaluating Bone Biomaterials Mediated Osteogenesis

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Osteoblast lineage cells are direct effector cells for osteogenesis and, therefore, commonly used to evaluate in vitro osteogenic capacity of bone biomaterials. This strategy has achieved a degree of success when developing novel bone biomaterials; however, inconsistent results between in vitro and in vivo studies are not uncommon. Some candidate bone biomaterials developed applying this strategy are later found to perform below expectations in vivo without satisfying new bone regeneration, suggesting the mechanisms that govern the material's capacity to mediate osteogenesis requires further investigation. The emerging field of osteoimmunology and immunomodulation in osteogenesis has informed a paradigm shift in our view of bone biomaterials–from one of an inert to an immune-osteomodulatory material–highlighting the importance of immune cells in the material-mediating osteogenesis. Neglecting the importance of the immune response during this process may be a major shortcoming in the current assessment and could explain the inconsistence between in vitro and in vivo conditions. In this study we evaluated a angiogenic bone biomaterial cobalt incorporated β-tricalcium phosphate (CCP) using both a traditional and a novel approach to assess osteogenesis, the latter including the use of immune cells. It was found that CCP by itself was sufficient to enhance osteogenic differentiation of bone marrow stem cells, whereas this effect was attenuated when macrophages were involved. In response to CCP, macrophages switched M1 phenotype extreme, releasing pro-inflammatory cytokines and bone destructive factors. When CCP materials were implanted into a rat femur condyle defect model, there was an increase of inflammatory markers and bone destruction, coupled with fibrous encapsulation rather than new bone formation. These findings demonstrated that inclusion of immune cells (macrophages) in the in vitro assessment matched the in vivo tissue response, and provides a more accurate indication of the essential role of immune cells when assessing material-stimulated osteogenesis in vitro.

Session No.: Y11-02

Adult Human Neural Crest-derived Chondrocytes Are Potential Candidates for Treating Degenerative Disc Disease with an Autologous Cell-transplant Approach

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Nasal chondrocytes (NCs) of the nasal septum have an increased rate of proliferation and synthesis of proteoglycan and collagen in vitro in contrast to chondrocytes from articular cartilage. Moreover, adult human NCs display features of environmental plasticity as they partially adopt the HOX-expression profile of cells at a recipient implantation site in vivo. These traits are likely ascribed to the fact that NCs are derivatives of cranial neural crest stem cells of neuroectoderm origin as opposed to articular cartilages derived from the mesoderm. The previously mentioned characteristic of NCs and their uncomplicated accessibility have made them interesting to analyze as a potential cell source for regenerative medicine based orthopedic treatments. The intervertebral discs (IVD) are the largest non-vascularized structures of the body. Disc degeneration is a main causes for chronic back pain, as degeneration can structurally deform the disc, which can irritate or compress the spinal cord. In the past decade injection of autologous cells, mainly MSCs, into degenerative discs has been attempted as a treatment with limited success. This has been attributed to a low survival rate of the injected cells due to the harsh environment within the IVD, which is hypoxic, acidic, low in nutrients, and possibly inflamed in a degenerative state. Our findings indicate that NCs would be an optimal cell source for the treatment of disc degeneration disease as they are prone to survive and synthesize cartilaginous extracellular matrix in vitro in conditions resembling those of the IVD (i.e., low oxygen and low glucose concentration).

Session No.: Y11-03

Genipin-crosslinked Decellularized Annulus Fibrosus Matrix/Chitosan Scaffolds for the Culture of Annulus Fibrous-derived Stem Cells

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Background: While tissue engineering method has become an ideal approach for annulus fibrous (AF) regeneration recently, it remains challenging because of the heterogeneity of AF tissue. Decellularized matrix is a novel tissue-specific biomaterial for tissue engineering, yet lacks sufficient mechanical strength. In this study, we used Genipin to crosslink decellularized AF matrix (DAFM) and chitosan (CS) to fabricate biomimetic scaffolds for AF tissue engineering. Methods: Tensile tests, SEM and AFM were used to characterize DAFM samples. After being cultured on the scaffolds, the morphology, proliferation, gene expression, and cell traction forces (CTFs) of AF-derived stem cells (AFSCs) was examined. Results: Biomimetic DAFM-CS composites were crosslinked using Genipin to fabricate highly porous scaffolds of various elasticity (47, 75, and 120 kPa, respectively). After AFSCs were cultured on these scaffolds for 7 days, the gene expression and protein production of collagen-II and aggrecan decreased with the elasticity of scaffold, whereas the expression of collagen-I was exactly the opposite case. Similarly, the CTFs of AFSCs gradually decreased with the elasticity of scaffold. The morphology of cells was almost round on the scaffolds of low elasticity and spindle-like on the scaffolds of high elasticity. Discussion and conclusion: DAFM/CS composite scaffolds with different elasticity were fabricated using Genipin-crosslinking. These scaffolds mimicked the outer, middle and inner zones of native AF tissue and markedly affected the differentiation of AFSCs. Importantly, the substrate elasticity-dependent changes (cell morphology, gene expression, and CTF) of AFSCs were similar to the cellular, mechanical and biochemical characteristics of cells from outer region to inner region of native AF tissue.

Session No.: Y11-04

Lumbar Intervertebral Disc Allograft Transplantation: Healing and Remodeling of the Bony Structure

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Previous human study suggested that fresh-frozen intervertebral disc allograft transplantation can relieve neurological symptoms and restore segmental kinematics. Before wide clinical application, researches into the pathophysiology of the postoperative disc allograft are needed. One important question that remains to be answered in disc allografting is the healing process of the host-graft interface and the subsequent change of the endplates. With the goat model for lumbar disc allografting, histology, micro-CT analysis, SEM and EDX mapping, were applied to evaluate the healing of the host-graft interfaces, the remodelling of subchondral bone, the changes of the bony and cartilaginous endplates after transplantation. It was found that healing of the host-graft interfaces started at 1.5 months and completed at 6 months by natural remodelling. This bony remodelling was also noted in the subchondral bone area after 6 months. The bony endplate was well preserved initially, but gradually replaced by trabecular bone afterwards; on the other hand, the cartilaginous endplate became atrophic at 6 months and nearly disappeared at the final follow-up. Collectively, after intervertebral disc allograft transplantation, bony healing and remodelling were seen which ensured the stability and mobility of the disc-transplanted segment, but the integrity of bony and cartilaginous endplates was gradually lost and nearly disappeared finally.

Session No.: Y11-05

Mesenchymal Stromal Cells Regulate Host Cell Mobility and Immune Response During Osteogenic Differentiation

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Introduction: Cell-cell interaction is believed to play a critical role in the cell-based therapy for bone regeneration. However, the mechanisms involved in the interaction between donor cells and host cells during the bone healing process are still not clear. This study investigated the potential effect of vascular endothelial growth factor (VEGF) produced by osteogenically differentiated mesenchymal stromal cells (O-MSCs) on the recruitment and regulation of un-differentiated MSCs and macrophages during osteogenesis. Methods: Factors secreted from MSCs during osteogenic differentiation were monitored by cytokine arrays. Indirect co-culture models were applied to study the effect of VEGF derived from O-MSCs on the motility, cell morphology and migration-related gene expression in MSCs as well as the regulation of local immune response. A mouse skull defect model was used to unveil the cell recruitment, macrophage activity and new bone formation following the implantation of O-MSCs. Results: It was found that VEGF secretion increased dramatically when MSCs were subjected to osteogenic differentiation. The secreted VEGF by O-MSCs stimulated the recruitment of MSCs and macrophages to the bone defects. It was noted that O-MSCs could regulate the local inflammation by modulating the expression of pro-inflammatory cytokines in macrophages. Furthermore, neutralization of OMSC-secreted VEGF led to a significant decrease of cell recruitment, cytokine secretion and new bone formation. Conclusions: This study demonstrated that VEGF secreted by O-MSCs plays a pivotal role in the cell recruitment and regulation of local immune response during osteogenesis.

Session No.: Y11-06

Shelf Life Evaluation of Chondrogenic Induced Aged Adult Stem Cells Stimulated with bFGF

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Great deal of interest has been generated by the potential therapeutic applications of adult stem cells in diseases; but there remain obstacles regarding to cell number, viability and delivery time. Harvesting and proliferating cells from older adults for autologous therapy can be challenging. Cryopreservation protocols include potentially toxic additives that prevent direct use, hence there is the need to develop relatively simple methods to process and distribute cells for human applications. ADSCs and BMSCs were harvested from 30 patients, aged 76 ± 8yrs. Cells were proliferated with basal medium and several concentrations of bFGF. They were induced to chondrogenic lineage and furthermore stored at 40C in different solutions for 120 hrs in order to evaluate their shell life. Cell counts, viabilities and specific proteins were evaluated. Results from culturing with basal medium revealed that stem cells stagnate, produced multi pseudopodia and enlarged vacuole with PDT of 144 ± 16hrs. When bFGF was added at concentrations of (10, 15, 20 and 25) ng/ml, cells revived to proliferation. Multiple pseudopodia turned into daughter cells divisions. PDT were significantly increased to (42 ± 8hrs, 36 ± 12hrs, 24 ± 6hrs and 23 ± 4hrs) respectively ($p<0.05$). On shell life evaluation, both cells maintained counts and viabilities greater than 70% in F12 DMEM +10% serum and in human serum, but not in PBS and normal saline. With PBS, their viabilities fall below 70% in 48hrs and in normal saline 24hrs. The chondrogenic markers, Sox9, Col II, and Aggrecan were detected on cells cultured in F12 DMEM and autologous serum after 120hrs, but vanished from DPBS and normal saline after 72hrs. A concentration of (10 – 20) ng/ml of bFGF boosted the proliferation of aged adult cells and the delivery time for implantation can be up to 120 hrs in serum FD medium at

Session No.: Y11-07

Modulation of Focal Adhesion Dynamics and Maturation by Nanospaced Adhesive Peptide Domains Regulates Rho/Rac Signalling in Mesenchymal Stem Cell

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The ability of mesenchymal stem cells to sense changes in extracellular matrix (ECM) in their microenvironment is crucial to their survival. Transmembrane receptors can bind the peptide sequences within ECM that permit cells to assess the mechanical properties of ECM, and respond by recruiting intracellular proteins to form focal adhesions. Our previous data showed that increased lateral nanospacing (30 to 60 nm) of arginine-glycine-aspartate (RGD) peptides led to lower cell spread areas and less mature FA formation in human mesenchymal stem cells (hMSCs). However, the exact underlying mechanism driving these substantial differences remains unclear. In this work, we created tailored surfaces with controlled lateral RGD spacing (~30 and 60nm) on self-assembled PEO-N3 nanodomains. Fluorescence resonance energy transfer (FRET) reporters were utilized in hMSCs to investigate the molecular effectors and regulators involved in mechanotransductive signaling, including vinculin, FAK, Src, RhoA and Rac 1. We observed that smaller nanospacing resulted in lower vinculin tension sensor (VinTS) FRET activity, indicating the development of higher levels of tension at the site of focal adhesions compared to larger nanodomain spacings. By combining fluorescence-lifetime imaging microscopy and VinTS FRET, we observed that the recruited vinculin also remained in these FAs for longer at smaller nanodomain spacings, with fluorescence life times of up to τ=2.78ns, compared to τ=2.26ns on larger nanodomain spacings. More FAK-GFP biosensor was colocalized at FA sites with longer FA lifetime on smaller nanodomain spacing when compared to larger nanospacing, suggesting the formation of more mature FAs. In terms of modulation of intracellular signaling pathways associated with mechanotransduction, higher RhoA and Src FRET activity and lower Rac FRET activity were noted when the lateral spacing of peptides was decreased from 60nm to 30nm. This study shows that smaller lateral nanospacing of adhesion peptides enables the recruitment of greater numbers FA proteins, enabling the

Session No.: Y11-08

Conjugated Polymer Nanodots as Ultrastable Long-term Trackers to Understand Mesenchymal Stem Cell Therapy in Skin Regeneration

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Stem cell–based therapies hold great promise in providing desirable solutions for diseases that cannot be effectively cured by conventional therapies. To maximize the therapeutic potentials, advanced cell tracking probes are essential to understand the fate of transplanted stem cells without impairing their properties. Herein, conjugated polymer (CP) nanodots are introduced as noninvasive fluorescent trackers with high brightness and low cytotoxicity for tracking of mesenchymal stem cells (MSCs) to reveal their in vivo behaviors. As compared to the most widely used commercial quantum dot tracker, CP nanodots show significantly better long-term tracking ability without compromising the features of MSCs in terms of proliferation, migration, differentiation, and secretome. Fluorescence imaging of tissue sections from full-thickness skin wound–bearing mice transplanted with CP nanodot-labeled MSCs suggests that paracrine signaling of the MSCs residing in the regenerated dermis is the predominant contribution to promote skin regeneration, accompanied with a small fraction of endothelial differentiation. The promising results indicate that CP nanodots could be used as next generation of fluorescent trackers to reveal the currently ambiguous mechanisms in stem cell therapies through a facile and effective approach.

Session No.: Y11-09

Bioinformatic Analysis of Lesioned Sciatic Nerve Following Nerve Crush

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The peripheral nervous system (PNS), unlike the central nervous system (CNS), has an intrinsic capacity for self-repair and regeneration. However, for severe peripheral nerve injuries, the self healing capacity of peripheral nerve is normally insufficient for satisfactory functional restoration. Therefore, gaining a better understanding of the cellular and molecular mechanisms underlying peripheral nerve injury is of vital importance and may benefit the development of its clinical treatment. In the current study, we used a rat model of sciatic nerve crush injury and gathered nerve samples from adult rats underthough surgery for 0, 1, 4, 7, and 14 days. Transcriptome deep sequencing and bioinformatic analysis were performed to characterize the global transcriptional changes at each time point following nerve crush. Our outcomes suggested that genes related to inflammation and immune response were significantly elevated at 1 day post nerve injury and were slightly down-regulated, but still higher than the 0 day control, during the whole repairing stage. Genes related to cellular growth and proliferation followed the same trend while genes related to cellular movement and development kept up-regulated during regeneration. The development of hematological system and the synthesis of lipid were involved in the later stage of nerve regeneration, contributing to the rebuild of nerve and blood vessel. These outcomes revealed the molecular mechanisms underlying peripheral nerve regeneration from the perspective of gene regulation, and thus might help to identify brand-new clinical targets for the treatment of nerve injury.

Session No.: Y11-10

Induction of Tendon Stem Cells Toward Tenogenic Differentiation and Tendon Formation by Uniaxial Mechanical Loadings

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Background and Aims: Tendon injury is one of the most common musculoskeletal injuries and causes pain, discomfort or even disability. Tendons are mechanosensitive fibro connective tissue but little is known about the role of mechanical loading in tendon development, homeostasis and regeneration. In this study, we compare the impact of uniaxial and biaxial mechanical stimulation on tendon derived stem cells isolated from mouse and human. Method: Tendon derived stem cells (TDSC) were isolated from human and mice tendon tissue and characterized by flow cytometry, colony unit formation and multipotent differentiation assay. Uniaxial mechanical stimulation of tendon-like tissue in programmable bioreactor (up to 0.25Hz for 8h/day) or biaxial mechanical stimulation of tendon-derived stem cells in Flexcell system were used directly to cells or tissue. Quantitative real-time polymerase chain reaction, western blot analysis, histology and immunohistochemistry and immunofluorescence confocal microscopy were used to investigate the formation of tendon-like tissue. Results: We have shown that tenogenic differentiation and tendon formation can be induced by programmable uniaxial mechanical stimulation in bioreactors without growth factors. 6% cyclic tensile strain (0.25Hz, 8h/d) in the uniaxial loading bioreactor can induce tendon formation but suppressed osteogenic, adipogenic or chondrogenic differentiation. In contrast, using Flexcell system we showed that biaxial mechanical stimulation induces TDSC toward osteogenic rather than tenogenic differentiation (6% strain, 0.25Hz, 8h/d). Uniaxial mechanical stimulation induces activation of the AKT signaling pathway but reduce in phosphorylation of beta-catenin (Ser552). The tendon-like tissue formation induced by uniaxial mechanical stimulation in the bioreactor exhibited a tendon-specific microstructure, protein expression and gene profile of tendon tissue. Conclusion: Our study demonstrated that uniaxial mechanical stimulation mimics the physiological conditions for tenogenic differentiation and development. Our study has highlighted the importance of applying appropriate mechanical stimulation when dissecting the molecular pathways involved in tendon development and function.

Session No.: Y11-11

Incorporation of Stromal Cell-derived Factor-1α in Silk Fibroin-bacterial Cellulose Membrane Enhances the Pregnancy Rate of Injured Uterine Through the Promotion of Endometrial and Vascular Regeneration

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Severe infection and mechanical injury in the uterus may lead to infertility and miscarriages. As there is a lack of effective treatments for functional repair of uterine injury, this study aimed to develop a chemotactic composite by silk fibroin-bacterial cellulose (SF-BC) membrane loaded with recombinant human stromal cell-derived factor-1α (rhSDF-1α) to address this problem. Rat model of uterine damage was divided into three groups and implanted with nothing, SF-BC only and SF-BC loaded rhSDF-1α, respectively. The regeneration effects of three groups were tested and compared. Results showed that SF-BC loaded rhSDF-1α could promote the endometrial regeneration and arteriogenesis and enhance pregnancy outcomes of the injured rat uterus. These results indicated that the chemotactic composite developed here is promising for functional uterine repair and regeneration.

Session No.: Y12-01

Reconstruction of Large Segmental Bone Defects in Sheep Tibiae Using Novel Baghdadite Scaffolds as Bone Graft Substitutes

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Significant clinical challenges have been encountered in the effective long-term treatment of large bone defects arising from traumatic injury or tumour resection. Such defects, in particular those involving segmental bone loss, often fail to achieve satisfactory healing from conventional treatment methods such as bone grafting. This study reports the evaluation of novel ceramic scaffolds when implanted as bone graft substitutes in a clinically relevant in vivo model. We have previously developed a bioactive ceramic named baghdadite (Ca3ZrSi2O9), which can be fabricated into highly porous scaffolds and further modified with a nanocomposite coating layer composed of polycaprolactone and bioactive glass nanoparticles to improve toughness. These baghdadite scaffolds have exhibited promising biological and mechanical properties for use as bone graft substitutes in our previous investigations in vitro and in a rabbit model. In this study, we evaluate the efficacy of using baghdadite scaffolds, unmodified or modified with the nanocomposite coating, to induce the healing of critical-sized segmental bone defects in sheep tibiae over 26 weeks. Radiographic, biomechanical, micro-CT and histological analyses showed that both unmodified and modified baghdadite scaffolds were able to withstand physiological loads at the defect site, and induced substantial bone formation in the absence of supplementation with cells or growth factors. Notably, all samples showed significant bridging of the critical-sized defect (average 80%) with evidence of bone infiltration and remodelling within the scaffold implant. The unmodified and modified baghdadite scaffolds achieved similar outcomes of defect repair, although the latter had an initial mechanical advantage due to the nanocomposite coating. These results support the potential use of baghdadite scaffolds as purely synthetic bone graft substitutes to augment the reconstruction of large bone defects while circumventing the drawbacks of autografts and allografts.

Session No.: Y12-02

Modulation of Human Stem Cell Behaviour Using Binary Colloidal Crystals

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The control of stem cell behaviour on biomaterial surfaces is the key to a broad range of biomedical applications. The specific surface properties of biomaterials such as nanotopography can profoundly influence cell morphology, proliferation, and differentiation. It provides a more controllable way to direct stem cells than chemical induction. Recently, we have established a group of elaborate surfaces to display ordered topographies with tuneable chemistry called self-assembled monolayer binary colloidal crystals (BCCs), and introduced them as substrates for cell culture. The BCCs were fabricated using evaporation induced colloidal self-assembly (EICSA). Four BCCs (5Si-PMMA, 5Si-PS, 2Si-PMMA, and 2Si-PS) with different sizes (i.e. 5 μm Si and 2 μm Si) with different surface chemistries (SiO2, PS and PMMA) were selected. Cellular responses of human mesenchymal and pluripotent stem cells (MSCs and PSCs) on BCCs were investigated in terms of adhesion, proliferation, and differentiation. Immunostaining, qPCR, and FACS at different time points were used to study different cell-BCC interactions. In general, stem cells didn't like the surface properties of BCCs resulting in a smaller spreading area compared with flat controls. This change leads a higher expression of chondrogenic and osteogenic markers of human MSCs without chemical induction. Furthermore, human ESCs (H9) expressed higher pluripotent markers on BCCs compared to flat control. BCCs also improved the cell reprogramming generating better iPSC colonies. This study established a new platform for cell culture, and demonstrated that the BCCs are promising in controlling stem cell behaviour in desired way. BCCs can be used as next generation cell culture tools.

Session No.: Y12-04

Generation of Tissue-engineered Small Intestine with an Enteric Nervous System Derived Exclusively from Human Pluripotent Stem Cells

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BACKGROUND: Loss of intestine from infection, ischemia, or injury can lead to the inability to absorb enough nutrients to sustain life, a condition called short bowel syndrome (SBS). Chronic medical management and surgical interventions provide little success in increasing intestinal function. Given the high rates of morbidity and mortality in SBS patients, development of patient-specific intestinal tissue for transplantation is essential. We have generated human intestinal organoid-derived tissue-engineered small intestine (HIO-TESI) from embryonic stem cells. However, HIO-TESI fails to develop an enteric nervous system (ENS). The purpose of our study is to establish an ENS in HIO-TESI derived exclusively from human pluripotent stem cells (hPSCs). METHODS: H9 hESCs or WTC hiPSCs were maintained in mTeSR. H9 cells were temporally exposed to Activin A, CHIR99021, and FGF4 as previously described to generate HIOs. Enteric neural crest (ENC) progenitor cells were derived from hPSCs exposed to LDN193189, SB431542, CHIR99021 and Retinoic Acid as previously described. HIOs and unsorted ENC progenitor cells were seeded onto biodegradable scaffolds, wrapped in the omentum of adult NSG mice, and allowed to mature for 3 months. ENS-HIO-TESI was immunostained for neuronal, glial, and smooth muscle cell markers: EDNRB, RET, TrkC, Tuj1, GFAP, s100b, ChAT, Calbindin, Calretinin and SMA. RESULTS: H&E analysis of ENS-HIO-TESI demonstrates mature villus formation with the presence of underlying smooth muscle and ganglia within the submucosal and muscular layers. Excitatory neurons (Tuj1/ChAT/Calretinin or Tuj1/ChAT/Calbindin), glia (GFAP/s100b) and ganglia (EDNRB/RET/TrkC) were identified. CONCLUSION: ENC progenitors give rise to excitatory neurons, glia and ganglia within the submucosal and muscular layers of ENS-HIO-TESI. These data demonstrate establishment of an ENS in HIO-TESI supplemented with ENC progenitor cells derived solely from hPSCs.

Session No.: Y12-05

Multimodality Noninvasive Imaging for Assessing Therapeutic Effects of Exogenously Transplanted Cell Aggregates Capable of Angiogenesis on Acute Myocardial Infarction

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Although the induction of neovascularization by cell-based approaches has demonstrated substantial potential in treating myocardial infarction (MI), the process of cell-mediated angiogenesis and its correlation with therapeutic mechanisms of cardiac repair remain elusive. In this work, three-dimensional (3D) aggregates of human umbilical vein endothelial cells (HUVECs) and cord-blood mesenchymal stem cells (cbMSCs) are constructed using a methylcellulose hydrogel system. By maximizing cell-cell and cell-ECM communications and establishing a hypoxic microenvironment in their inner cores, these cell aggregates are capable of forming widespread tubular networks together with the angiogenic marker αvβ3 integrin; they secret multiple pro-angiogenic, pro-survival, and mobilizing factors when grown on Matrigel. The aggregates of HUVECs/cbMSCs are exogenously engrafted into the peri-infarct zones of rats with MI via direct local injection. Multimodality noninvasive imaging techniques, including positron emission tomography, single photon emission computed tomography, and echocardiography, are employed to monitor serially the beneficial effects of cell therapy on angiogenesis, blood perfusion, and global/regional ventricular function, respectively. The myocardial perfusion is correlated with ventricular contractility, demonstrating that the recovery of blood perfusion helps to restore regional cardiac function, leading to the improvement in global ventricular performance. These experimental data reveal the efficacy of the exogenous transplantation of 3D cell aggregates after MI and elucidate the mechanism of cell-mediated therapeutic angiogenesis for cardiac repair.

Session No.: Y12-06

Hypoxia Precondition Cultured Medium of Stem Cell Enhance Wound Healing Through Hif-1α/VEGF Pathway in STZ-induced Diabetic Rats Model.

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Purpose: Studies indicated mesenchymal stem cells (MSCs), derived from bone marrow or adipose tissue etc. could enhance diabetic wound healing in rats. However, clinical applications in wound healing are still concerned under FDA situation. We investigated whether cultured medium of adipose-derived stem cells (ASCs) under hypoxia-precondition could enhance diabetic wound healing in a STZ-induced diabetic rodent mode Materials and methods: Rat diabetes would be induced in male Wistar rats (weigh: 300-350 g) by a single injection of streptozotocin (STZ) (65 mg/kg i.p.). Rat dorsum was shaved and a 6×5-cm dorsal skin was drawn on the rats as animal model followed our previous report. Diabetic rats received different dosage of hypoxia conditioned medium in wounding bed post-operatively. The expression levels of the growth factors and related-cytokines were evaluated. Results: The results revealed the morphological changes of ASCs did not show obvious difference between human and rat ASCs under hypoxia and normoxia conditioning. VEGF and related cytokine were significant increased in hypoxia group, as compared to that in normoxia. HIF-1 expression of nuclear extracts of rat ASCs revealed significant increase. Our in vivo animal study showed hypoxia-induced ASC-conditioned culture medium could enhance diabetic wound healing as compared to that in controls in a STZ‐induced diabetes rodent dorsal wounding model. Conclusion: Hypoxia-induced ASC-conditioned culture medium could enhance diabetic wound healing and should not one option for critical wound healing.

Session No.: Y12-07

Determining the Impact of Existing Guidelines and Guidance in the Reporting of the Use of Adipose Derived Stem Cells in Humans and Animals

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Along with the rising interest in sourcing stem cells from the adipose tissue, there is also an increasing concern that the term adipose tissue-derived stem cell (ASC) is inappropriately used to refer to the adipose stromal vascular fraction (SVF). To determine the impact in reporting, we reviewed 116 published reports on the application of ASC in humans and animals based on the 2013 published International Federation for Adipose Therapeutics and Science (IFATS)/ International Society for Cellular Therapy (ISCT) joint statement. Parameters related to ethics, safety and study design were also evaluated based on the existing guidelines in the use of stem cells for clinical trials and preclinical studies. It is disconcerting that 4 among the 47 papers or 8.51% (CI 2.37–20.38) surveyed after publication of IFATS/ISCT statement reported using ASCs but in fact they used unexpanded cells. When we compared how reporting fared between papers on application of ASCs in humans and in animals ($p<0.001$), reporting on human applications tend to misreport SVF as ASCs despite the published guidance. Only 28/47 or 59.57% (CI 44.27–73.63) explicitly reported that adherent cells were used, while 35/47 or 74.47% (CI 59.65–86.06) identified expression of surface markers, and 25/47 or 53.19% (CI 14.72–30.65) verified the multi-lineage potential of the cells. While there are a number of papers examined in this survey that were not able to provide adequate information on the characteristics of ASCs used and with some erroneously referring to the SVF as stem cells, there are more room for improvement in the quality of reporting in the application of ASCs in humans and animals. As more research is required to fully understand the therapeutic potential, effectiveness and safety of ASCs, a unified global effort to comply with existing guidelines would definitely provide rapid and reliable results.

Session No.: Y12-08

A Fiber-progressive-engagement Model to Evaluate the Composition, Microstructure, and Nonlinear Pseudoelastic Behavior of Porcine Arteries and Decellularized Derivatives

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Background: The theoretical fiber-progressive-engagement model was proposed to describe the pseudoelastic behavior of an artery pre- and post-decellularization treatments. Material and methods: Native porcine arteries were harvested and decellularized with 0.05% trypsin for 12 h. The uniaxial tensile test data was fitted to the fiber-progressive-engagement model proposed herein. The effects of decellularization on the morphology, structural characteristics, and composition of vessel walls were studied. Results: The experimental stress-strain curve was fitted to the model in the longitudinal and circumferential direction, which demonstrated the adequacy of the proposed model ($R2 > 0.99$). The initial and turning strains were similar in the longitudinal and circumferential directions in the aorta, suggesting the occurrence of collagen conjugation in both directions. Discrepancies in the initial and turning strain and initial and stiff modulus in both directions in the coronary artery revealed the anisotropic features of this vessel. Decellularization induced a decrease in the initial and turning strains, a slight change in the initial modulus, and a substantial decrease in the stiffness modulus. The decrease in the initial and turning strain can be attributed to the loss of waviness of collagen bundles because of the considerable decrease in elastin and Glycosaminoglycan GAG contents. Conclusions: This simple non-linear model can be used to determine the fiber modulus and waviness degree of vascular tissue. Based on these results, this mechanical test can be used as a screening tool for the selection of an optimized decellularization protocol for arterial tissues.

Session No.: Y12-09

Design and Fabrication of Sialostent for the Treatment of Ductal Stenosis Under Sialoendoscopy

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Salivary duct strictures are the second most frequent cause of obstructive sialadenitis and frequently involve the parotid ductal system and mainly affect females. Strictures are usually a result of epithelial duct injuries following recurrent infections or traumas caused by sialoliths or surgical procedures, although congenital strictures have also been described. Sialendoscopy offers a minimally invasive approach to salivary duct stenosis. This technique allows endoscopic intraluminal visualization and offers a better way to treat strictures of the ductal system, ultimately reducing or eliminating the need for sialadenectomy and obviating related surgical risks. The use of postoperative stenting and corticosteroid injection through a stent or duct to prevent stricture or stenosis are recommended. There are no clear guidelines for stent insertion, however, it is generally agreed that stenting is necessary after stricture dilation or if significant ductal trauma was encountered during stone removal. Many researchers have reported the results using various types of salivary duct stents with different outcomes. We believe that the result of our study which revealed the outcome of stent was related to the different material.The findings might provide valuable information for future design of salivary duct stents. In this study, we tried to use biodegradable material of polyester type, including polylactic acid (PLA),poly L-lactide(PLLA), poly ε-capolacton(PCL), poly(butyl-co-succinyl) adipate, and it is coated with hyaluronic acid(HA), collagen, or gamma-glutamic acid(γ-PGA) for design of salivary stent which may have intensity and flexibility. The inner diameter of the fabricated tube was 1.3 mm which was suitable for implantation via sialendoscpy. To compare with commercial stent, the fabricated stent is biodegradable with highly mechanical force to support. Preliminary data showed that the fabricated stent could be put into the salivary duct of animal model and we hope the design can be used for human in the future.

Session No.: Y12-10

Lactoferrin-conjugated Gold Nanoparticle for Targeting Glioblastoma via Oral Delivery

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Gold nanoparticle as a therapeutic delivery carrier has been reported in various application due to its physical properties. If gold nanoparticle can be orally absorbed, its application may be extended. Unfortunately, it has low absorption efficiency from the gastrointestinal (GI) tract to bloodstream. Therefore, to overcome its limitation orally, here we newly synthesized lactoferrin-conjugated gold nanoparticles (AuNP). In general, lactoferrin receptor is highly expressed on the small intestinal epithelial cell. So, we adapted nano-sized AuNP modified with the lactoferrin, glutathione and polyethylene glycols (PEG) for preparation of long-circulating AuNP with improved half-life. We evaluated the physicochemical properties of lactoferrin conjugated AuNP with ICP-MS, HR-TEM, UV-Vis spectrophotometer and so on. When lactoferrin-conjugated AuNP was orally administered into the mice, we determined high concentration of lactoferrin-conjugated AuNP in bloodstream, which was quantitatively measured by ICP-MS. Thus we confirmed the conjugation ratio of each compartment in our definitive synthesized AuNP-GSH-PEG-Lf particle by UV-visible spectrometry and BCA assay. However, in the case of oral administration of AuNP with lactoferrin conjugation, lower amount of them was detected. Interestingly, it is known that glioblastoma in brain cancer highly express lactoferrin receptor. Therefore, it is possible that the orally absorbed lactoferrin-conjugated AuNP can be targeted to the glioblastoma. As ongoing experiments, we are in vivo doing the effect of lactoferrin-conjugated AuNP via photo thermal therapy (PTT) in the glioblastoma orthotopic animal model

Session No.: P1-001

Generation of Stem Cells-derived Cardiac Sheets Through Coculture and Transfer-printing

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Coculturing stem cells with the desired cell type is an effective method to promote the differentiation of stem cells into the desired cell type. The features of membrane used for coculture are crucial to achieving the best outcome. Not only should the membrane act as a physical barrier that prevents the mixing of the cocultured cell populations, but it should also allow effective interactions between the cocultured cells. Unfortunately, conventional membranes used for coculturing do not sufficiently meet the requirements. In addition, cell harvesting using proteolytic enzymes following coculture impairs the cell viability and extracellular matrix (ECM) produced by the cultured cells. To overcome these problems, we developed a nano-thin and highly porous (NTHP) membrane with a thermoresponsive property for the generation of transfer-printable sheets of cells differentiated from stem cells through coculture. The NTHP membrane is ~20-fold thinner (380 nm in thickness) and ~27-fold more porous (54% in porosity) than the conventional membrane. Coculturing with this membrane not only enhanced stem cell differentiation compared to conventional coculture methods, likely through effective physical contacts between the cocultured cells and the fast diffusion of bioactive molecules across the membrane, but also efficiently generated cell-separated, ECM-preserved, highly viable, and transfer-printable sheets of the differentiated cells. The coculture platform developed in this study may be effective for producing therapeutic multilayered sheets of various types of cells that are differentiated from stem cells through coculture.

Session No.: P1-002

Bio-inspired Carbon Nanoparticles for Cell-guiding and Biosensing Uses

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Carbon nanoparticles (CNPs) have shown their great potential in many applications, including diagnostic probes, nontoxic labeling, drug carriers, hydrogen generation, specific catalysts, and so on. Due to CNPs having graphite-like multilayers, nanoscale sizes, and fluorescence, they can be relatively useful in biomedical applications to replace other fluorescent dyes and inorganic quantum dots; however, fluorescent dyes always undergo bleaching in a long-term irradiation and inorganic quantum dots usually comprise heavy metal and toxic elements. In this study, we prepared some bio-inspired carbon nanoparticles that exhibit high fluorescence quantum yields, good conductivity, excellent dispersion in aqueous solution, high cell-uptake efficiency, and less cytotoxicity as well. We were inspired from mussels' adhesive components to synthesize polydopamine nanoparticles and then employed carbonization process to prepare fluorescent CNPs. Using some surfactants we could control the sizes of CNPs and increase their dispersion in water. Characterization of CNPs and investigation of their special physical properties was carried out for biomedical uses. XRD patterns revealed the graphite structure of the polydopamine-prepared CNPs, and their FTIR and UV-vis spectra showed that the carbonized polydopamine nanoparticles retained polypeptide structures. Fluorescence spectroscopy was used to confirm the excitation of CNPs at 360 nm and the emission of blue light with >20% of quantum yield. TEM was used to recognize the sizes of nanoparticles and prove that the addition of surfactants could downsize particle to several nanometers. The fluorescent CNPs were applied to stain cells in vitro without any harmful effect on L929 fibroblast cells. Due to the polydopamine-derived CNPs having good electrical conductivity, we manipulated some cell uptake with conductive CNPs to observe the influence of cell attachment and growth under an electric field. The CNPs were also sensitive to some metal ions with surface chelation effect to decrease fluorescence intensity, which display a promising potential for biosensors.

Session No.: P1-003

Reconstruction of Endometrium-like Tissue Using Cell Sheet Engineering

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The uterus is one of the female reproductive organs and plays a crucial role in mammalian fertility. However, the endometrial disorders are one of the causes that led to female infertility. The goal of this study is to establish a technique to fabricate and transplant cultured endometrial stromal cells (EnSCs) sheets for endometrial reconstruction using a cell sheet tissue engineering approach to regenerate damaged tissue. A blowing film machine was used to produce thin (19.12±3.17 μm) and transparent (81±4%) polyvinylidene fluoride (PVDF) membranes. Secondly, on the basis of plasma chemistry, a two-step method was established for the grafting of a disulfide bond-containing amino acid followed by a biopolymer on transparent PVDF membranes to achieve a detaching system between disulfide bonds which can be cleaved via reduction using an amino acidic reductant. After atmospheric environmental plasma treatment and interfacial polymerization, the poly γ-glutamic acid (γ-PGA) or hyaluronic acid-grafted PVDF surface became more hydrophilic and the water contact angle of γ-PGA-grafted PVDF decreased from 73.9±2.39˚ to 33.8±4.58˚ while the contact angle for hyaluronic acid-grafted PVDF decreased from 73.9±2.39˚ to 47.8±4.29˚. Thirdly, mouse EnSCs were cultured on modified PVDF membranes. After cell growth reached confluence, cell cultures were detached as a laminated sheet by reductant additives. The characteristics of mouse EnSC sheets were further analyzed and evaluated through histology and immunohistochemistry stain. The mouse EnSCs' viability in the obtained cell sheet was confirmed by WST-1 assay and vital assay. The results demonstrate that EnSCs could be cultured on both of the γ-PGA and hyaluronic acid-grafted PVDF membrane, and then be further detached from the dish surface into complete cell sheets. In conclusion, this system gives a new approach towards generating cell sheets for regenerating damaged tissue and can contribute to future applications in endometrial regenerative therapy.

Session No.: P1-004

Reconstruction of Rabbit Corneal Endothelium with Cell Sheet Engineering

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Human corneal endothelial cells have no proliferative capacity in vivo. When the cell density drops below a critical level, the corneal endothelial cells can no longer pump enough water to compensate for diffusion into the cornea, resulting in stromal edema, corneal clouding and eventual vision loss. However, access to donated tissue is limited worldwide resulting in critical need for new sources of corneal grafts. The number of such grafts produced by each donor eye could be increased significantly if corneal endothelial cells were expanded in culture before grafting. In this study, we developed a novel therapy technique to fabricate and transplant cultured rabbit corneal endothelial cell sheets for corneal endothelial reconstruction. On the basis of plasma chemistry, we have designed a two-step method to prepare a poly γ-glutamic acid (γ-PGA)-grafted polyvinylidene fluoride (PVDF) thin film. After plasma treatment and interfacial polymerization, the γ-PGA-grafted PVDF surface became more hydrophilic and the water contact angle decreased from 74.0˚ ± 2.40 ˚ to 37.8 ˚ ± 1.32 ˚. XPS analysis of the γ-PGA-grafted PVDF showed an increase in relative atomic% of nitrogen, oxygen and sulfur. NIH-3T3 and Rabbit corneal endothelial cells were seeded onto the modified PVDF thin film, and confluent cells were detached as a laminated sheet by reductant additive with cleaving the disulfide crosslinkage to thiol groups. The cell numbers were measured by using WST-1 cell proliferation assay kit. Immunohistochemical staining verified physiologically phenotypic expressions of ZO-1 and Na+/K+ ATPase. Furthermore, the formation of a continuous monolayer of RCECs detached from the γ-PGA-grafted PVDF thin film was confirmed by H&E staining. We hope this work may be an optimized method to fabricate bioengineered corneal endothelium and show potential to facilitate CEC transplantation in the future.

Session No.: P1-005

Characterization and Uptake Mechanisms of Gelatin Nanoparticles in Human Corneal Epithelial Cell Layers and Rabbit Cornea

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Eye-drop is a common formulation in ophthalmology, but only 5 % of the administered dose retained in the eye after 5 min. In order to develop an effective drug carrier for ocular drug delivery, charged gelatin nanoparticles (GPs) were made and evaluated with respect to particle size, surface charge, morphology, and in vitro examination with human corneal epithelial cell (HCTs). A rabbit model was used to test the particles distribution in cornea. The GPs prepared using type A gelatin were positively charged (GP(+), +33 mV; size, 180.6 ± 45.7 nm). Water-soluble tetrazolium salt (WST)-1 assay showed that GPs (+) were nontoxic to HCE cells. The uptake mechanism of HCTs with fluorescence GPs was used. The internalization of GPs in HCTs was confirmed by confocal laser scanning microscopy analysis, pointing out the high retention of GPs on HCTs. The transport mechanism for paracellular pathway was examined by transepithelial electrical resistance (TEER) measurements, Western blotting. The GPs could be uptake into HCTs without influence the zonula occludens-1 (ZO-1) expression of confluent HCTs layer showing no risk for open tight junction of HCTs layer. In vivo examination showed no serious irritation to the rabbit eyes. Corneal cryosections showed widely distributed fluorescent nanocarriers, from the anterior to the posterior part of the cornea of the GP (+) group, and higher fluorescence intensity in the GP (+) group was also observed. Overall, GPs as cationic colloidal carriers was efficiently uptake by HCTs and adhesive on the cornea without irritating the eyes of the rabbits and can be retained in the cornea for a longer time. Thus, cationic GPs have a great potential as carriers for ocular drug delivery.

Session No.: P1-006

Multiphoton-based Artificial Cell Niche Platform for Cell Matrix Interaction Study

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Cell niche is the microenvironment in which cell survivals are supported and cell fates are determined. In details, niche can provide cell with biochemical signals and mechanical signals which cell can sense and thus response. Artificial cell niche can help us understand the cellular interaction between cells and the microenvironment directly. In order to mimic the biochemical cues in biological system, extracellular matrix proteins were crosslinked to the surface of BSA microstructures with two photon excitation based fabrication technology. In the meantime, mechanical properties, such as elastic modulus, is controlled by the crosslinking laser power and scan cycle. Immunostaining of integrin was used to demonstrate the cell and niche interaction. This work presents an easy and smart way to spatially control the heterogeneity in artificial cell niche study.

Session No.: P1-007

Recapitulation of Mesenchymal Condensations in Collagen-based Microencapsulation

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Formation of mesenchymal condensations is an important process in endochondral ossification. However, current in vitro models for it such as pellet and micromass cultures are not able to manipulate the cells' specific 3D matrix environment, which is important in researching cell-ECM interaction in cartilage tissue engineering. Self-assembled collagen-encapsulated microspheres have been shown to provide a collagen fibril meshwork for the cells. Additionally, the microspheres contract post-encapsulation, which may induce early signaling cues towards chondrogenesis. This study investigated whether or not collagen microencapsulation recapitulated early mesenchymal condensations, if the process provided any cues to aid future chondrogenic induction, and attempted to determine the apt time for chondrogenic induction. hMSCs were encapsulated in 3D collagen microspheres and the constructs were evaluated at various early time points for mesenchymal condensation markers. PNA, focal adhesion-related proteins, and early chondrogenic transcription factors and markers, were evaluated using staining. Gene expression of the latter was also evaluated using RT-PCR. Transient staining of mesenchymal condensation marker PNA was found. It was also observed that SOX9 and RUNX2 were upregulated until 24 hours. Clustering of integrins and focal adhesion proteins were detected. Fibronectin additionally had a transient deposition at a similar timeframe as the PNA staining. These results suggest that collagen microencapsulation simulates mesenchymal condensations to some extent and has potential to be used as an in vitro model to study mesenchymal condensations.

Session No.: P1-008

Manufacture of Uniform Pore Size 3D Scaffolds and the Influence of Pore Size Toward Adipose-derived Stem Cells Differentiation Ability

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3D scaffolds that were manufactured with former techniques perform a non-uniform pore size, which results in many factors that might affect cell behaviors. Therefore in this research, we apply microfluidic techniques and modify the radius of microfluidic channel to manufacture uniform-pore-sized 3D gelatin scaffolds with a variety of pore sizes. With these scaffolds, we start to compare the morphology of human adipose stem cells (hASC) under different pore sizes (50, 100 etc.) . It is shown that hASCs can stretch among adjacent bubbles in both scaffolds. But among the larger pore size scaffolds, more hASCs can grow in a single bubble. Furthermore, we compare the differentiation ability of hASC in these scaffolds applying chondrogenesis. Recent research shown that hASCs tend to aggregate during chondrogenesis. We suggest that pore size will have a significant impact towards the ability of hASC chondrogenic differentiation. Several tests and staining are made to compare the differentiation ability among different pore sizes.

Session No.: P1-009

Fabrication and Characterization of Porous Silk Fibroin /Gelatin/ Chitosan Scaffolds for Tissue Engineering Applications

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We prepared composite scaffolds with different blending ratios of silk fibroin/chitosan/gelatin. silk fibroin was extracted from the Bombyx mori silkworm. Silk fibroin, chitosan and gelatin mimic the properties of natural extra-cellular matrix. The porous scaffolds were fabricated by means of freeze-drying technique and characterized by ATR-FTIR,SEM, MTT-Assay, swelling properties, degradation rate and mechanical properties. Scanning electron microscopy showed the scaffolds of SF/CTS/G with proper pore sizes,good interconnectivity and porosity which is suitable for cell growth. The composite formation was confirmed using Fourier transform infrared and X-ray diffraction. The addition of gelatin increased water uptake and degradation rate and reduced mechanical strength but silk fibroin affect reversely on the degradation and mechanical strength of composite scaffolds. Biocompatibility of scaffolds was demonstrated by MTT-assay which lead to the growth and adhesion of endothelial and fibroblast cells. In this study, Fabricated composite scaffolds have the potential for tissue engineering applications.

Session No.: P1-010

Janus Membrane Functionalized with Hydrogel for Skin Regeneration

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Wound dressing has a pivotal role in wound treatment by absorbing exudate, allowing for air permeation, and protecting the wound sites from external harsh environment. Here, we developed hydrogel-immobilized Janus membrane and estimated it for usage as wound dressing. Hydrophobic and hydrophilic surfaces were rendered onto commercially used polyester fabric through initiated chemical vapor deposition (iCVD) process. Antibacterial property of hydrophobic surface was evaluated by bacterial growth reduction analysis. Its macroporous structure allowed for air permeation, but bidirectional water penetration was restricted due to the hydrophobic fluoropolymer coating layer. Hydrophilic surface that had carboxylic acid groups was functionalized with gelatin methacrylated (GelMA) hydrogel, which released vascular endotheilar growth factor (rh-VEGF) in controlled manner, for accelerated wound healing and skin regeneration. When the hydrogel-immobilized Janus membrane was applied to full thickness dorsal skin defect model, it prevented leakage of exudate and maintained optimal moist environment at wound by exudate evaporation. The GelMA hydrogel covered by this Janus membrane also enhanced the quality of regenerated skin with thick epidermal layer. In conclusion, the Janus membrane with GelMA hydrogel can be simply fabricated and utilized for various types of wounds based on its advanced functions.

Session No.: P1-011

Effect of Concentration, Ph and Temperature on the Physicochemical and Biological Properties of Self-assembly Acid-soluble and Pepsin-soluble Collagen Hydrogels Derived from Rabbit Epidermis

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Acid-soluble and pepsin-soluble collagens with triple helical structures were successfully extracted from the rabbit epidermal skin by two different extraction approaches. The kinetics of collagen self-assembly process modulated by concentration, pH value and temperature during fibril formation to form collagen hydrogels were investigated. The present study determined the relationship among fibril formation, microstructure and rheological properties of collagen hydrogels. Moreover, collagen hydrogels, where the mechanical properties are tuned by different extraction methods and conditions of fibril formation were used for biological evaluation. Using these collagen hydrogels, our results demonstrate the ability to de-couple matrix stiffness from matrix density and structure in collagen gels, and that increased matrix stiffness results in decreased sprouting and outgrowth of blood-derived endothelial colony-forming cells in vitro.

Session No.: P1-012

The Proliferation and Endothelial Differentiation of Human Adipose-derived Stem Cell on Zwitterionic Poly-SBMA Hydrogels

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Zwitterionic poly-sulfobetaine methacrylate (SBMA) has been well studied for its superhydrophilic and ultralow biofouling properties, making it to be a promising material for high biocompatibility. Adipose-tissue derived stem cells (ASCs), which are isolated from fat tissues, are easily obtainable and also can differentiate to multiple lineages, including adipogenesis, osteoblast and chondrocytes. Although pSBMA being ultralow biofouling material, it can be modified with peptides containing the amino acid sequence arginine-glycine-aspartic acid (Arg-Gly-Asp, RGD) to promote cell adhesion ability and QK, a de novo engineered VEGF mimicking peptide, to promote endothelial differentiation. In this study, we are seeding human adipose-tissue derived stem cells (hASCs) on different concentration RGD/QK-modified pSBMA hydrogels to observe cell adhesion ability and endothelial differentiation.

Session No.: P1-013

Bioadhesive Hydrogel Based on Catechol-modified Hyaluronic Acid for Regenerative Medicine

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Recently, with the development of surgical techniques, bioadhesive materials are adding appeal for numerous doctors and researchers. However, the synthetic materials like cyanoacrylates are generally dissolved in organic solvents and hence lead to allergic response, inflammation, and poor adhesion in the presence of biological fluids. Mussel adhesive proteins, secreted by M. edulis foot pad, are enriched in the post-translationally modified amino acid 3,4-dihydroxy-phenylalanine and can form strong covalent and noncovalent interactions with substrates. Hyaluronic acid, a glycosaminoglycan widely distributed in extracellular matrix, has good biocompatibility, and play an important role in the body's biological development and repair process. In this experiment, we succeeded conjugating the catechol groups to the backbone of hyaluronic acid, which can cross-link and form hydrogel within several minutes, and exhibit good biocompatibility and bioadhesion. To obtained catechol modified HA with high degree of substitution, dopamine is coupled to the carboxyl group of HA in organic phase. Since catechol can be easily oxidized to quinone and then cross-linked under neutral to basic condition, we form HA-catechol hydrogel in PBS with a little amount of oxidant. Due to the catechol-amino/thiol reaction, the hydrogel matrix can express high interaction with free amino and thiol groups in proteins. After 14d incubation, BSA loaded in MeHA hydrogel is continuously released to 80%, whereas 70% of BSA in HA-catechol has been trapped in the gel matrix. HA-catechol hydrogel can also sequester the proteins and peptides from surroundings and enhance cell adhesion and spreading. For the cell encapsulation, the live/dead staining and Alamar blue assay shows the HA-catechol hydrogel does not exhibit obvious cytotoxicity. Moreover, according to lap-shear test, both ex situ and in situ formed HA-catechol hydrogel perform excellent adhesive properties. Hence, this bioadhesive hydrogel shows good potential as tissue engineering scaffold for cartilage repair, or as growth factors loaded wound dressings.

Session No.: P1-014

Characterization of Copper-based Nanoparticles with Different Synthesis Conditions and the Application to Photothermal Therapy

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Photothermal therapy (PTT) is a new technique combining nanotechnology and biomedical engineering for cancer treatment. As other metallic nanoparticles (NPs), copper nanoparticles (CuNPs) have unique optical characteristics. An adsorption peak of CuNPs was observed in adsorption spectra, indicating that CuNPs will exhibit localized surface plasmon resonance (LSPR) effect, which means energy of photon at a wavelength of 590 nm can be transferred to the free electron on NPs. The energy of the resonance cause a temperature elevation. This phenomenon can be used to kill cancer cells. However, due to the instability, CuNPs were not widely used as a PTT agent because they may induce reactive oxygen species (ROS) generation and cause cytotoxicity. This drawback is needed to be controlled if we want to introduce CuNPs.to biomedical application. In the work, we synthesized CuNPs with polymer shell via one-step hydrothermal reduction reaction. The formation of Cu@polymer NPs is sensitive to the presence of halide ions in the reaction. Next, we developed a smooth oxidation process of Cu@polymer NPs to fabricate polymer surface coating-Cu2O shell-Cu core nanocomposite. UV-visible spectra determined the as-prepared Cu@Cu2O@polymer NPs with absorption band covered from red to near infrared (NIR) wavelengths. It indicates that the LSPR effect of Cu@Cu2O@polymer NPs can be stimulated by NIR light, which has higher penetration to tissue and is more appropriate for light-induced therapy. Also, these nanoparticles exhibited optical and physical stability in the aqueous solution. Moreover, compared with Cu@polymer NPs, less ROS generation and cytotoxicity were induced while cells were incubated with Cu@Cu2O@polymer NPs. The cell death pathway attributed to NP-cell interaction was also studied. The photothermal effect was examined by light stimulation. Cu@Cu2O@polymer NPs or Cu@polymer NPs are more appropriate to be applied in PTT therapy will be indicated.

Session No.: P1-015

Development of New Triphasic Scaffold for Osteochondral Tissue Engineering Using 3D Printing Technology

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INTRODUCTION: A number of studies of cell therapy and tissue engineering using 3D printed scaffolds for mimicking the structure of bone or cartilage have been reported recently. However these tissue engineering scaffolds have substantial limitations in the excessive growth of bone tissue and the weak adhesion between bone and cartilage layer. To improve osteochondral scaffolds, we developed a three layered scaffold which consisted of a bone layer, an intermediate layer and a cartilage layer to produce an artificial osteochondral composite tissue that could be applied to a living body. METHODS: Three layered osteochondral scaffold consisted of cartilage, intermediate and bone layers were prepared by using 3D printing technology. Cartilage and bone layers were fabricated by porcine cartilage derived atelocollagen-containing ECM and hydroxyapatite-containing PCL respectively. Intermediate layer was a compact layer composed of a mixture of materials for cartilage and bone layers. Experimental and control groups were divided into scaffolds with and without intermediate layer, respectively. After seeding rabbit chondrocytes to the cartilage layer, scaffold from all groups were cultured for 1 week in vitro and then implanted into the osteochondral defect of rabbit femoral condyle to evaluate the tissue formation in vivo. RESULTS: In histological examination (H&E, safranin-O), chondrocytes and a partial formation of cartilage tissue were observed after 1 week cultured in vitro, and well developed cartilage and bone tissues were observed in the implanted scaffolds from experimental group after 4weeks. Micro-CT observation showed that there was less excessive growth of bone tissue in the experimental group. DISCUSSION: The cartilage layer in knee joint of rabbit was too thin and was not suitable for this study. However, in this study, we could see the feasibility of 3D printed 3 layered scaffolds. And it is necessary to obtain more accurate results by a test using bigger animals.

Session No.: P1-016

Electroactive Biodegradable Polyurethane Significantly Enhanced Schwann Cells Myelination for Peripheral Nerve Tissue Engineering

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Peripheral nerve injury usually results in the devastating and permanent loss of sensory and motor function, causing the decrease in life quality of patients. For the regeneration of injured peripheral nervous system (PNS), Schwann cells (SCs) play a critical role during this period because the matured myelinating SCs can release various neurotrophins to attract injured neurons and aid axon elongation. However, although many techniques have been introduced to enhance SCs' myelination through via micro RNA modification or complex external stimuli such as electrical stimulation, it remains an ongoing challenge to develop a simple and effective method to induce SCs' myelination and promote their responses to peripheral injured nerve. Here we present a biomaterials-approach via a novel conductive biodegradable polyurethane based on poly(glycerol sebacate)-co-aniline pentamer (PGSAP) polymer to significantly enhance SCs' myelin gene expressions and neurotrophin secretion for peripheral nerve regeneration. The PGSAP co-polymers are synthesized via the incorporation of PGS and different content of conductive aniline pentamer (AP), and the conducting PGSAP-H polyurethane films have been easily prepared by crosslinking PGSAP with hexamethylene diisocyanate (HDI). These polyurethane films with different AP contents perform highly tunable mechanical properties, biodegradable behavior and electroactivity. SCs are cultured on these conductive polymer films, and the biocompatibility of these films and their ability to enhance myelin gene expressions and sustained neurotrophin secretion are successfully demonstrated. The mechanism of SCs' neurotrophin secretion on conductive films is demonstrated by investigating the relationship between intracellular Ca2+ level and SCs' myelination. Furthermore, the neurite growth and elongation of PC12 cells are induced by adding the neurotrophin medium suspension produced from SCs-laden conductive films. These data suggest that these conductive degradable polyurethanes that enhance SCs' myelin gene expressions and sustained neurotrophin secretion perform great potential for nerve regeneration applications.

Session No.: P1-017

Physical Properties and Regeneration Rate Correlation of Photocurable Biodegradable Co-polymer: Selection of Materials for Tissue Engineering

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As the applications of biodegradable polymer become more common in tissue engineering, a wide range of biodegradable polymers became available over the last two decades. As most traditional biodegradable polymers were thermoplastic polymeric materials, the fabrication of biodegradable scaffolds and devices were limited to thermal molding. Meanwhile, many of the existing biodegradable polymers suffer from a short half-life due to rapid degradation upon implantation, exceedingly high stiffness, and limited ability to functionalize the surface with chemical moieties. With the high mechanical strength, most common biodegradable polymers became useful in bone tissue engineering. However, the overall stiff mechanical properties leads to the lack of choice of material for soft tissue engineering. In this work, the combination between three biodegradable polymers is explored through photocuring: polycaprolactone (PCL), poly(ethylene glycol) (PEG) and poly(glycerol-sebacate) (PGS). The various combination of co-polymer ratio led to products with Young's modulus ranging between 0.5-10 MPa, right around the general soft tissue mechanical properties. Through the analysis of substrate stiffness, ultimate tensile strength (UTS), elongation at break and the degradation properties, several combinations are identified as useful materials for heart, lung, liver, kidney, and vasculature regeneration. With the photocuring capabilities, the co-polymer exhibits great devices and scaffold formation capabilities. Preliminary animal testing also proved that the co-polymers were non-toxic with minimum immunoresponses.

Session No.: P1-018

Nanofiber Yarn/Hydrogel Core-shell Scaffolds Mimicking Native Skeletal Muscle Tissue for Guiding 3D Cell Alignment, Elongation and Myogenic Differentiation

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Skeletal muscles are the mainly responsible tissues in human body for generating forces and facilitating voluntary movement. However, under compromised conditions, severe trauma would cause the loss of muscle functionality and these injuries are irreversible. Designing scaffolds that can mimic native skeletal muscle tissue and induce 3D cellular alignment and elongated myotube formation remains an ongoing challenge for skeletal muscle tissue engineering. Herein, we present a simple technique to generate core-shell composite scaffolds for mimicking native skeletal muscle structure through the combination of electrospinning and photocrosslinking process, which comprise the aligned nanofiber yarns (NFYs) core and the photocurable hydrogel shell. The poly-carprolactone (PCL), silk fibroin (SF) and polyaniline (PANI) were blended together to prepare the aligned NFYs by a developed dry-wet electrospinning method. The poly(ethylene glycol)-co-poly(glycerol sebacate) (PEGS-M) polymer, a good biocompatible photocurable hydrogel material, was chosen to prepare the hydrogel shell due to its ability to encapsulate cells for a long-term cultivation. Then a series of core-shell column and sheet scaffolds were fabricated by encapsulating aligned NFYs cores within hydrogel shell after photocrosslinking. C2C12 myoblasts are seeded within the core-shell scaffolds, and the good biocompatibility of these scaffolds and their ability to induce 3D cellular alignment and elongation are successfully demonstrated. Furthermore, the 3D elongated myotubes formation within core-shell scaffolds are also performed after a long-term cultivation. These data suggest that these core-shell scaffolds combining of the aligned NFYs core that can guide the myoblasts alignment and differentiation, and the hydrogel shell that can provide a suitable 3D environment for nutrition exchange and mechanical protection, will have a great potential for skeletal muscle tissue engineering applications.

Session No.: P1-019

Integrated Bi-Phasic Composite Scaffold with Mineralized Collagen Sponge and Hyaluronan-Chitosan Hydrogel for Osteochondral Regeneration

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Osteochondral defect indicates the injury to both the articular cartilage and the underlying subchondral bone. In order to repair an osteochondral defect, the needs of the bone, cartilage and the bone-cartilage interface must be jointly taken into consideration. An integrated but heterogeneous composite construct is regarded as promising scaffold for osteochondral graft. In this study, osteochondral substituent (OCS) is developed and consists of two distinct but integrated layers corresponding to the cartilage and bone components. In the upper layer, a biocompatible in situ forming hydrogel composed of N, O-carboxymethyl chitosan (NOCC) and aldehyde-functionalized hyaluronic acid (AHA) demonstrates similar mechanical, swelling, and lubricating behavior to articular cartilage. In the lower layer, a mineralized sponge scaffold with hydroxyapatite and type I collagen displays porous architecture, osteoconductive, and osteoinductive properties to generate new bone. Two cell types (osteoblast and chondrocyte) were respectively seeded into individual layer of OCS for in vitro chondrogenesis and osteogenesis investigations. After 21 days culture, the integrity of OCS could not only be maintained but also showed good cell viability and proliferation over time. Moreover, OCS could significantly promote tissue regeneration when compared to the single phase scaffold because of hierarchical structure and cellular coculture synergistic effect. It is noteworthy to mention that an integrated bi-phasic OCS scaffold provides a complete transition between the bone and the cartilage layers without requiring extra manipulative procedures during implantation; thus, simplifying clinical applications. In conclusion, the OCS can effectively promote osteochondral tissue regeneration in vitro and has great potential for tissue engineering.

Session No.: P1-020

Fabrication of a Sandwich Construct Scaffold by Silk Fibroin/Bacterial Cellulose-Blended Sponge and Electrospun Membrane for Potential Rotator Cuff Tendon Tear(RCTT) Tissue Engineering

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Silk fibroin (SF) is a protein produced by several insects and spiders. Silkworm fibroin that is usually isolated from the silk produced by Bombyx mori silkworms can be processed to obtain films, nonwoven nets and sponges. SF has been widely studied as the scaffold for ligament tissue engineering due to its biocompatibility, slow degradability, and remarkable mechanical properties. In this study, we fabricate a sandwich construct patch combining with two aligned fibrous membranes and a porous sponge scaffold in between. The porous sponge scaffold as fabricated by adjusting a proper ratio of bacterial cellulose (BC) and silk fibroin (SF). Aligned fibrous membranes will be fabricated by electrospinning with oriented structure to enhance the mechanical strength and to guide the cell differentiation. In this study, the mechanical properties of both SF/BC sponges and electrospun membranes and the sandwich construct were evaluated. The morphology of them are illustrated by SEM pictures. The performance of the sandwich construct in cell culture of 3T3 fibroblasts and tenocytes are investigated for possible applications in rotator cuff tendon healing.

Session No.: P1-021

The Analysis of Keratins Incorparated with Chitosan and Its Application in Bone Tissue Repairing

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Keratin has intrinsic bioactivity, biodegradability, and biocompatibility. In the other hand, issue of bone repair clinically is very popular, especially regeneration of large traumatic bone defects. Therefore, using of regenerative biomaterials has been extensively studied for bone tissue engineering. In this study we used keratin as biomaterial base, and mixed with chitosan to enhance the mechanical strength. We will analyze the properties of the composites Keratin/Chitosan, and prove that the following films and scaffolds are suitable for the adhesion and proliferation of porcine adipose stem cells (pASCs) , and most importantly, the differentiation to osteoblast. We hope this technique could be applied to the repair and regeneration of the damaging bone tissue in the future.

Session No.: P1-022

Electroconductive and Biocompatible Carbon Nanotube/Polyurethane/Collagen Scaffolds for Cardiac Tissue Engineering

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cardiac tissue engineering improves cardiac functions by cells, scaffolds and engineering strategies. To mimic the native myocardial environment, carbon nanotube as conductive material and collagen as a natural polymer was used in composites to obtain electroactive and biocompatible scaffolds. Scaffolds were fabricated based on polyurethane, collagen and polyurethane/collagen, polyurethane/carbon nanotube and polyurethane/ collagen/carbon nanotube composites by electrospining and sprey methods. carbon nanotubes were added to polymer solution and then sonicated for 5 h at room temperature to obtained a homogenous suspension and nanofibrous scaffolds were then prepared. Aligned and random polyurethane were also fabricated as controls. The scaffolds were seeded with H9c2 cardiac myoblasts and cultured for up to 8 days to investigate cell viability, adhesion and morphology in scaffolds without electrical stimulation. the metabolic activity of cells in polyurethane/ collagen/carbon nanotube composites was significantly higher than other scaffolds. With increasing the concentration of collagen within the composites, the fiber diameters and elasticity of scaffolds decreased and biocompatibility increased. mechanical properties of scaffolds were increased by polyurethane component. polyurethane/ collagen/carbon nanotube composites had excellent electrical properties. our findings indicated that the incorporation of carbon nanotube and collagen into nanofibrous scaffolds provided electrically conductive and biocompatible constructs for cardiac tissue.

Session No.: P1-023

Biomimetic and Bioresponsive Hydrogel Encapsulated with Polyelectrolyte Complex Nanoparticles for Sustainable Controlled Delivery of Multiple Growth Factors

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Neural stem cells (NSCs) provide potential therapeutic strategy for neural tissue repair after traumatic injury, stroke and degenerative neural disease. However, poor regenerative capability of NSCs in the lesion site due to the inflammatory response, loss of structural support and trophic factors limits their therapeutic efficacy. Normal extracellular matrix (ECM) is constructed by several molecules such as hyaluronic acid (HA) and proteoglycans which provide an ideal microenvironment for binding and stabilization of growth factors, chemokines and other ECM proteins from degradation by proteinases and serve as a niche for promoting NSC proliferation in vivo. In this study, we specifically develop an ECM-mimetic hydrogel composed of high molecular weight HA and glycosaminoglycan-based polyelectrolyte complex nanoparticles (PCNs) to deliver stromal cell-derived factor-1α (SDF-1α) and fibroblast growth factor-2 (FGF-2) in response to matrix metalloproteinase (MMP) activity within the injured site. SDF-1α bounded on heparan sulfate is known to optimize SDF-1α presentation and to recruit endogenous NSCs to the wound area. On the other hand, continuous expression of FGF-2 that is complexed with chondroitin sulfate plays a critical role in regulating injury-induced NSC proliferation and differentiation. The protection and sustainable controlled delivery of both growth factors are achieved by the conjugation of MMP-cleavable and MMP-inactive peptides linked on the PCNs in HA hydrogel. The combination of growth factor-loaded PCNs and biodegradable hydrogel is expected to enhance chemotactic recruitment and survival of endogenous NSCs and provide a stem cell niche to promote NSC proliferation and differentiation for neural tissue repair and regeneration.

Session No.: P1-024

Oxygen Clustering in Graphene Oxide Is Responsible for Its Fluorescence Property

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Graphene oxide (GO) can be modified for several biology applications due to its large surface area, flexibility, hydrophilicity and dispersibility in aqueous solutions. Here, we present a simple method to modify GO, with no chemical treatments, to produce blue fluorescence by oxygen clustering and the remaining graphic properties. The modified-GO has the potential for using in cellular imaging and except to track the relative position between materials and cells. Our simple method offers a suitable way to tune and enhance the fluorescence property of GO, which creates opportunities for various future applications.

Session No.: P1-025

Preparation of Extracellular Matrix Developed by the Use of Porcine Articular Cartilage and Possibility Study as an Anti-adhesive Film

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After abdominal surgery, many people undergo intra-tissue adhesion. Articular cartilage is a useful biomaterial on account of emerging extracellular matrix and non-collagenous tissue components. In this study, we supposed porcine articular cartilage (PAC) is a valuable material as an anti-adhesive barrier. PAC film which was made of prepared PAC powder for anti-adhesive barrier was difficult to control mechanical properties. To improve mechanical properties, we cross-linked PAC film (Cx-PAC film) and treated by heating. The Cx-PAC film showed varying enthalpies, tensile strengths and contact angles before and after thermal treatment. This result exhibited we controlled mechanical properties. Next, we examined the anti-adhesive properties with scanning electron microscopy, fluorescence image and MTT assay by using human umbilical vein endothelial cells (HUVECs). HUVECs were adhered to the surface the plate and proliferated. On the other hand, Cx-PAC and thermal-treated Cx-PAC films revealed little or no cell attachment and proliferation. In conclusion, we suggested that the Cx-PAC film can be useful anti-adhesive barrier with controllable mechanical properties.

Session No.: P1-026

To Fabricate the Endothelized Autologous Vascular Graft by Biotube and Adipose Stem Cells (ADSCs) in Vivo

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Nowadays, the commercial synthetic vascular graft made by including Dacron and ePTFE for small diameter vascular (<6mm), with limited reendotheliaztion and less compliant, often result in thrombosis and intimal hyperplasia again. Even though, the use of autologous artery and vein also had the problem of size-mismatched to do the fistula or cardiovascular bypass surgery on the clinical. Therefore, small-diameter self-growing vascular grafts have been proposed.

Biotube is an in vivo tissue engineered approached to grow autologous graft by implanting a rod subcutaneously. In this study, we embedded 2mm-diameter silicone rod into New Zealand white rabbits dorsal subcutaneous to grow biotubes for 4weeks. Then, we focused on accessing the formation of functional endothelium alignment on the inner wall surface by seeding with adipose stem cells (ADSCs). The ADSCs-seeding biotube was implanted into the carotid aorta of rabbit for more than 1 amonth. The patency rates are observed by angiography, and the remodeling of endothelial cells by the fluorescence staining, including endothelia cell marker of CD31 and vWF, smooth muscle cell marker of α-SMA and Myosin Heavy Chain. On the other hands, Van Gieson was also investigated for conllagen fibers and elastin fibers. Last, the mechanical property by material test system was used to evaluate their function.

The results showed biotube could withstand the blood pressure in vivo. On the other hand, the ADSCs started differentiated into endothelia cell in the inner wall surface for 1 month. Moreover, ADSCs-seeding biotube showed with patency for 5 months. The fluorescence staining results, including CD31 and α-SMA showed that ADSCs not only differentiated into the endothelia cell but also smooth muscle cell, which fabricated the complete construction of small sized vascular graft. It could be speculated that the ADSC-seeded small sized biotube vascular could decrease the rate of intimal hyperplasia for implanting the longer time.

Session No.: P1-027

Improvement of Macrophage-constructed Microenvironments by Chitosan Degradation Products Facilitates Peripheral Nerve Regeneration

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Chitosan-based artificial nerve grafts have been widely employed to repair peripheral nerve defects and exhibit good clinical achievements. However, the underlying mechanisms are still not fully elucidated. In the present study, we observed that some pro-inflammatory cytokines were transiently up-regulated at the injury sciatic nerves bridged by silicon tubes filled with COS, the degradation products of chitosan. Meanwhile, a large number of macrophages were observed at the injury sites. Co-culture experiments revealed that COS-mediated macrophage migration required SCs. Based upon the transcriptome and gene regulatory network analysis, chemokines CCL2 and CCL7 and their respective regulatory miRNAs were identified as the potential targets of COS in SCs. The luciferase assay together with cell migration assay showed that the miR-327/CCL2 axis in the SCs is a downstream effector of COS. Furthermore, the immunohistochemistry confirmed that the SC-expressed CCL2 was up-regulated by COS in the injured sciatic nerves. Collectively, our results revealed a new molecular mechanism underlying the COS-promoted peripheral nerve regeneration. COS stimulates CCL2 secretion in SCs by down-regulating miR327, which induces macrophage migration to the injury sites to re-construct microenvironments and facilitates nerve regeneration. Our data provide a theoretical basis for the clinical application of chitosan-based grafts in peripheral nerve regeneration.

Session No.: P1-028

Periodical Assessment of Electrophysiological Recovery Following Sciatic Nerve Crush via Surface Stimulation in Rats

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When evaluating peripheral nerve regeneration, electrophysiological test is recognized as an optimal assessment, which is a quantitative, objective, and direct evidence reflecting function as compared to morphological examinations. In murine models of nerve regeneration, however, it remains a challenge to record compound muscle action potentials (CMAPs) periodically and non-invasively, i.e., with no insult to the nerve. In the present study, we recorded CMAPs in the gastrocnemius muscle weekly until 8 weeks after sciatic nerve crush by stimulating the nerve in a surface manner, and the electric stimuli were delivered to the skin between ischial tuberosity and major trochanter using bipolar hook electrodes. The CMAPs were reproducibly recorded in this way from 3 weeks post-injury, and both amplitude and latency were well correlated to postoperative time. Furthermore, a strong positive correlation was observed between CMAP amplitude and sciatic function index (SFI), a well-recognized assessment for sciatic nerve function. CMAP recordings by direct nerve stimulation at 8 weeks post-injury showed no significant difference in amplitude compared to surface stimulation, but the peak latency was relatively longer than the latter. This study indicated that non-invasive surface stimulation-based periodical recording of CMAPs was a practical electrophysiological approach to monitor the progression of peripheral nerve regeneration in murine models.

Session No.: P1-029

Repair of Rat Sciatic Nerve Defect by Using Allogeneic Bone Marrow Mononuclear Cells Combined with Chitosan/Silk Fibroin Scaffold

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Tissue engineered nerve graft (TENG), which composed of seed/support cells and biomaterial scaffold constitute a promising alternative to autologous nerve graft that have been the gold standard for the repair of peripheral nerve injury. Owe to the advantages of bone marrow mononuclear cells (BMMNCs) and chitosan/ silk fibroin (SF) scaffold, allogeneic BMMNCs were jointly used as a TENG for repairing rat 10-mm long sciatic nerve defect in our work. For the fate of BMMNCs, GFP-BMMNCs were traced at 1d, 7d, 10d and 14d after transplantation. And immunohistochemistry with neurofilament-200 was applied at 1 and 2 weeks to compare the velocity of regenerated nerve fibers which help understand how this TENG encouraged peripheral nerve regeneration. At 12 weeks, behavior observation, electrophysiological and histological assessments were performed to assess the outcomes of nerve regeneration and function recovery. All the results collectively demonstrated that the structural and functional recovery of the injured sciatic nerve and target muscle repaired by TENG was close to that by autograft, and better than that by chitosan/ silk fibroin scaffold alone. Therefore, introduction of BMMNCs to a chitosan/ silk fibroin scaffold yielded an improved outcome for repair and rehabilitation of peripheral nerves.

Session No.: P1-030

TGF-b1 Is Critical for Wallerian Degeneration After Rat Sciatic Nerve Injury

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Wallerian degeneration (WD) is a process of axonal degeneration distal to the injury site followed by a robust regenerative response. It involves degeneration and regeneration which can be directly induced by nerve injury and activated by transcription factors. Although WD has been studied extensively, the precise mechanisms of transcription factors regulating WD are still elusive. In this study, we reported the effect of transforming growth factor-b1 (TGF-b1) on WD after rat sciatic nerve injury. The data showed that TGF-b1 may express in injured rat sciatic nerve and cultured Schwann cells (SCs). Knock down of TGF-b1 expressions resulted in the reduction of SC proliferation and apoptosis, up regulation of cytokines and Smad2, 4. Enhanced expression of TGF-b1 could promote SC proliferation and apoptosis, down regulation of cytokines and Smad2, 4. Altered expressions of TGF-b1 may affect Smad and AKT but not c-Jun and extracellular regulated protein kinase (ERK) pathways. Our results revealed the role of TGF-b1 on WD and provided the basis for the molecular mechanisms of TGF-b1-regulated nerve degeneration and/or regeneration.

Session No.: P1-031

Glycated Wound Healing Model Using a Tissue-engineered Skin Made of Collagen and Chitosan to Study the Impact of Glycation.

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The wound healing process is essential to restore the skin barrier function to prevent fluid loss and infection. The peripheral nervous system plays an important role in this process by inducing neurogenic inflammation. Diabetes can cause neuropathy that aggravates wound healing closure, leading to ulcer development and high risks of lower limb amputation. This process is mainly due to diabetic-induced hyperglycemia causing tissue glycation and advanced glycated endproduct (AGE) formation such as N-carboxymethyl-lysine. Our hypothesis is that the deleterious effects of AGEs in wound healing which are observed in diabetes could be reversed by a treatment with anti-AGE and AGE-breaker molecules. Project's aim: Develop an in vitro wound within glycated tissue-engineered reconstructed skin to evaluate the influence of AGEs on the reepithelialisation process and to develop a treatment targeting AGEs to improve it. Method: We developed an in vitro wound-healing model by using a biomaterial made of collagen and chitosan to create a reconstructed skin in which an 8mm diameter wound is created at the epidermal level. The treatment of this model with glyoxal started one week before the wound was made and was maintained for three weeks to induce expression of AGEs and recapitulate the glycation process. We have evaluated the effect of a treatment with aminoguanidin, an anti-AGE molecule, and alagebrium, an AGE-breaker molecule, to establish if these molecules could improve different parameters in glycated wound healing. Results and conclusion: Results suggest that the tissue engineered skin we used is a good model to recapitulate the diabetic wound healing process and suggest that the action of aminoguanidin and alagebrium inhibits AGEs production and improves the reepithelialisation of the wound in glycated models. Thus, their topical application on ulcers could be a valuable approach to improve wound healing for diabetic patients with minimal systemic side effects.

Session No.: P1-032

Directing Tenogenesis of Stem Cells with Bioactive Nanofibers

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Directing Tenogenesis of Stem Cells with bioactive Nanofibers Zhang Can1，Zhang Erchen1，Yang Long1，Chen Xiao1，Ouyang Hongwei1 1.Dr. Li Dak Sum & Yip Yio Chin Center for Stem Cells and Regenerative medicine, School of Medicine, Zhejiang University, Hang Zhou, PR China Objective： Biophysical cues are often used to guide cells to orientate, which is also reported to modulate the physiology state of cells. Here, we Here, we examined the effect of small molecule modified scaffolds to direct tendon progenitor/stem cells (TSPCs) differentiate into tendons in vitro and in vivo. Method： The histone deacetylation inhibitor TSA were combined on the aligned nanofibers. TSPCs obtained from mouse were seeded onto the aligned and modified aligned nanofibers. The gene expression of tendon related genes were examined. Confocal observation was further performed. A rat Achilles tendon defect model was created and implanted with aligned or composite scaffold in vivo. The morphology of repaired tissues were analyzed by histological examination and transmission electron microscope. Mechanical testing was performed for mechanical properties. Results：Gene expression profile showed that composite aligned group upregulated the tendon related genes compared to the align group. Scx-GFP as a report system also showed that TSPCs cultured on the composite aligned scaffold maintain strong GFP fluorescence and cells exhibited spindle-shaped morphology, in contrast to weak GFP fluorescence on aligned scaffold. In rat Achilles tendon repair model, composite aligned scaffold treated tendon had superior structural and mechanical properties than aligned scaffold treated tendon. These findings present a strategy combining well-aligned fiber scaffold with small molecule for tendon regeneration and may assist in clinical regenerative medicine to treat tendon diseases.

Session No.: P1-033

Anatomy of Supraspinatus Insertion and Biomechanical and Histological Properties of Supraspinatus Enthesis in the Rat

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Background: With a similar shoulder anatomy between human and rat, the rat supraspinatus (SS) model has been used previously and demonstrated to be valid for the study of rotator cuff disease. In rotator cuff repair, a thorough understanding from SS insertion to deep SS enthesis is important for accurate reconstruction and preclinical bench studies; however, to our knowledge, no published evidence has been found to provide this basic information of rat SS. The study aims to anatomically delineate SS insertion and to qualitative and quantitative of SS enthesis in the rat. M&M: Nine adult male Sprague-Dawley rats weighting 400-450 g underwent bilateral detachment of the supraspinatus tendon (n=18) and randomly used for anatomical characteristics measurement (n=10), biomechanical testing (n=6), and histology (n=2). Cortical layer thickness in the greater tubercle was determined by direct measurement under sagittal section. Results: The insertion of rat SS was approximately narrow rectangular with an anteroposterior length of 2.3±0.18 mm and an mediolateral width of 1.1±0.19 mm. Its calculated area was 2.5±0.6 mm2. The cortical layer thickness in the greater tubercle directly below SS insertion was 0.66±0.1 mm. The max load, stiffness, and max stress of rat SS enthesis was 23.38±6.33 N, 39.03±9.28 N/mm, and 7.9±2.44 MPa, respectively. Histologically, the SS enthesis exhibits gradients between SS tendon and humerus into four distinct zones: tendon, uncalcified and calcified fibrocartilage, and bone. Conclusions: This study provided basic information about rat SS insertion and enthesis which may be considered while intending to translate rat model into clinics.

Session No.: P2-034

Vesicle-like Nanospheres of Amorphous Calcium Phosphate: Sonochemical Synthesis Using Adenosine 5' -Triphosphate Disodium Salt and Their Application in Ph-responsive Drug Delivery

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A rapid and simple strategy is reported for the synthesis of amorphous calcium phosphate (ACP) vesicle-like nanospheres using adenosine 5’-triphosphate (ATP) disodium salt as a biocompatible phosphorus source and stabilizer by the sonochemical method in mixed solvents of water and ethylene glycol (EG). The ACP vesicle-like nanospheres are characterized by X-ray powder diffraction (XRD), Fourier transform infrared (FTIR) spectroscopy, UV/Vis absorption spectroscopy, thermogravimetric (TG) analysis, scanning electron microscopy (SEM) and transmission electron microscopy (TEM), dynamic light scattering (DLS) and Brunauer-Emmett-Teller (BET) nitrogen adsorption. The ACP vesicle-like nanospheres exhibit essentially inappreciable toxicity to the cells in vitro. Furthermore, the as-prepared ACP vesicle-like nanospheres can be used as the anticancer drug nanocarrier and have a pH-responsive drug release behaviour using doxorubicin (Dox) as a model drug. The ACP vesicle-like nanosphere drug delivery system exhibits a high ability to damage cancer cells, thus, is promising for the application in pH-responsive drug delivery.

Session No.: P2-035

Development of National Guideline of Tissue-engineered Products Quality Control

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Nowadays, the key trend of worldwide medicine development is using of tissue-engineered constructions with substantionally modified cells and tissues in therapy of serious, socially significant diseases. The leading countries in practical implementation of these products are USA, European Union and Japan. Cell therapy is the one of such innovative directions. There are more than 10 cell therapy products, that have been succesfully registered and applied in USA and EU. According to report of Ministry of Healthcare of the Russian Federation, 34 biomedical cell products (BCP) are developing now in our country. In this way, development of national guideline for quality control of BCP in Russia is one of the main tasks of regulatory organisations. The Federal Law "On biomedical cell products" which will regulate the development and application of products, including human cell lines, is discussed currently in State Duma. Adequate quality control of these products is necessary to minimize the risks of using BCP in clinical practice. Quality control of BCP is carried out by manufacturer in several stages – cell banks control, in-process control and control of finished product. For some BCP with very short shelf life (less than 15 days), some results of finished product control may be received after its application. Alternative quick methods of analysis can be used to solve this problem. Main quality indicators that should be controlled by manufacturer are identity, viability, activity, safety (absence of bacterial, viral and mycoplasma contamination) and tumorigenicity. Some organoleptic, physical, physico-chemical or biological tests (visible inclusions, pH, LAL-test) can be included. Design and methods of quality control may be different for various types of BCP. The key requirement for all approaches to the quality control of BCP is that they must provide safety and efficacy of manufactured product.

Session No.: P2-036

A Novel Hierarchical in-vitro Coculture Model of the Liver Microvasculature for Pancreatic Cancer Cells Adhesion Monitoring

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Both researchers and industries are in need of a reliable in-vitro model for cancer cell extravasation which could overcome the limit of in-vivo experiments. In that regard, we proposed an in-vitro model composed of endothelial cells, pericytes embedded in collagen gel and hepatocytes layers, stacked in an original hierarchical structure that mimics the in-vivo physiological situation. The introduced model aims to address the lack of in-vivo likeness of in-vitro models by allowing interactions between the different cells that compose the liver microvasculature. Firstly, we investigated the influence of the different cell types of our model and the importance of using a PDMS bottom culture for oxygenation in a thick hierarchical coculture. We were able to obtain continuous monolayer of endothelial cells in all conditions. The secretions of albumin and VEGF from hepatocytes were measured and the interactions between the cell layers were confirmed. The production of Albumin was increased in coculture, when using a PDMS bottom plate, compared to monoculture and the produced VEGF was shown to be consumed by the endothelial cells in their coculture with hepatocytes. Next, we designed a quantitative cancer cell adhesion assay. We found that the adhesion was significantly lower in the coculture, compared to the monoculture of endothelial cells. Immunostaining for several endothelial markers was performed in order to explain the change in cancer cells adhesion. It was found that inflammation markers where overexpressed in monoculture compared to coculture and that constitutive markers were on the opposite trend, suggesting that the newly designed coculture model reflects better the healthy tissues situation. Finally, we can conclude the necessity to use PDMS bottom plate for oxygenation of the hepatocytes and the benefits of hierarchical coculture composed of endothelial cells, embedded pericytes and hepatocytes for the study of the adhesion of cancer cells in the liver microvasculature.

Session No.: P2-037

Delivery of 3D Cellular Assembly via Surface Modified Thermally Expandable Hydrogel by Using Mussel Inspired Coating and ECM Molecules

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Formation and harvest of 3 dimensional (3D) cellular assembly mimicking natural tissue has been developed in regenerative medicine and drug development. Although there have been various approaches to harvest and deliver 3D cellular assembly, they showed some limitations. For example, 3D cell printing requires complex system and cell encapsulating hydrogel may inhibit cell-cell interaction. Mono-layered cell stacking method needs long process time that leads concern for cell viability. Hence, we developed new system which can deliver multiple-layered cell assembly with maintaining cell-cell interactions and extracellular matrix (ECM) via rapid and simple method by using thermally-expandable hydrogels. We firstly coated human dermal fibroblast with fibronectin (FN) and gelatin in a layer-by-layer form to induce rapid 3D assembly formation without laborious process. We then modified surface of Tetronic® based hydrogel by using mussel-inspired coating layer (polydopamine(PD)) and FN to support stable 3D assembly formation. We examined FN adsorption on the hydrogel surface via chemical bond analysis, showing C-S bond signal only after PD coating. The hydrogels expanded by 1.4 times as temperature decrease from 37℃ to 4℃. 3D assembly formed on the modified surface with 1 μg/ml of FN was harvested within 10 min mediated by hydrogel expansion with high efficiency (>90%). However, decreased translocation efficiency (less than 10%) was observed from the modified hydrogel with higher FN amount. We could control the layer number by changing cell seeding density, and intact ECM and secretion of bFGF were observed from harvested assembly. After translocation to subcutaneous region of mouse, we observed stable retention of transplanted cells for 14 days and induction of angiogenesis into the transplanted assembly. In conclusion, we achieved rapid and easy translocation of 3D cellular assembly with maintained functions which can be applied to further applications such as ex vivo drug screening and regenerative medicine.

Session No.: P2-038

Highly Motile Rhabdomyosarcoma Cell Disorder of Myoblast Tissue Structure via Disruption of Myoblast Alignment

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Rhabdomyosarcoma (RMS) is a highly malignant tumor-type of skeletal muscle origin, hallmarked by local invasion. Interaction between invasive tumor cells and normal cells plays a major role in tumor invasion and metastasis. Culturing tumor cells in a three-dimensional (3D) model can translate tumor malignancy relevant cell-cell interaction and enable efficient in vitro model for cancer drug screening. To mimic tumor heterogeneity in vitro, a co-culture system consisting of a malignant embryonal rhabdomyosarcoma (ERMS) cell line RD and a normal human skeletal muscle myoblast (HSMM) cell line was established by cell sheet technology. Various ratios of RDs to HSMMs were employed to understand the quantitative effect on intercellular interactions. Disruption of sheet structure was observed in heterogeneous cell sheets having a low ratio of RDs to HSMMs, whereas homogeneous HSMM or RD sheets maintained intact structure. Deeper exploration of dynamic tumor cell behavior inside HSMM sheets revealed that HSMMs alignment was disrupted by highly motile RDs. This study demonstrated that RMS cells are capable of compromising their surrounding environment through induced decay of HSMMs alignment in a cell-based 3D system. This suggests that muscle disruption might be a major consequence of RMS cell invasion into muscles, which could be a promising therapeutic target to preventing tumor invasion. Our future work will focus on applying this in vitro model to cancer drug screening.

Session No.: P2-039

Structured Adipose-derived Constructs Influence the Onset of Neovascularization

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In tissue injuries such as severe burns or chronic wounds, it is essential that vasculogenesis occurs in a timely manner in order to supply oxygen and nutrients and therefore circumvent tissue necrosis. As a possible mechanism to influence cell guidance, surface structure has demonstrated to have an effect on mobility, both in vitro and in vivo. Therefore, a construct with structures and mesenchymal stem cell (MSC) containing adipose derivatives has the potential to be angiogenic and control the onset of neovascularization. Fractionated murine adipose constructs were produced with a polydimethylsiloxane (PDMS) mold. Circular transplants with a 6mm radius and 0.4mm thickness were implanted into the dorsal skinfold chamber (DSC) of four groups of male B6 mice (n=5) as follows: (I) unstructured control, (II) unstructured basic construct (BC), (III) 5µm gridded BC, and (IV) 50µm gridded BC. Intravital microscopy (IVM) was used to observe the onset of neovascularization, and histological analysis was performed to characterize wound healing. Mouse adipose vascular fragments survived for several days under various in vitro conditions. In groups (II) and (III), neovascularization occurred as early as day 3 followed by an intense angiogenic reaction. Group (IV) was delayed and capillaries were only visible on day 7. Group (III) reached stabilization by day 21, whereas groups (II) and (IV) where still normalizing. H&E and Masson's Trichrome histology stainings confirmed these results. The use of a 5µm structured construct may not only help organize the drive of vessel growth, but also promote surrounding cell migration into the defect site. Using such constructs may serve as a simple and promising vascularization strategy for patients that lack tissue to transplant or chronic wounds. As adipose tissue contains MSCs, which have the potential to differentiate into a variety of cells under appropriate environments, it may be useful for several different organs.

Session No.: P2-041

Engineering of Axially Vascularized Bone Grafts Towards the Treatment of Avascular Bone Necrosis

Alexander Haumer[1], Tarek Ismail[1], Rik Osinga[1], Laurent Tchang[1], Atanas Todorov[1], Nadja Menzi[1], Rene Largo[1], Alexandre Kaempfen[1], Dirk Schaefer[1], Arnaud Scherberich[1], Ivan Martin[1]

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Engineering of axially vascularized bone grafts towards the treatment of avascular bone necrosis A Haumer 1, T Ismail 2, R Osinga 2 , L Tchang 2, A Todorov Jr. 1, N Allafi 1, N Menzi 1, RD Largo2, A Kaempfen 2, I Martin 1, DJ Schaefer 2 , A Scherberich 1,2 1Department of Biomedicine, University Hospital Basel, Switzerland,2Department of Plastic, Reconstructive, Aesthetic and Hand Surgery, University Hospital Basel, Switzerland Avascular bone necrosis (AVN) is a degenerative disease caused by impaired vascularization and cellular death of bone, leading to challenging clinical scenarios. To avoid the bottlenecks of standard of care autologous bone grafts, stromal vascular fraction (SVF) cells of human adipose tissue, which contains vascular and skeletal progenitors, have previously been used to engineer osteogenic and vasculogenic grafts. This study aims at combining such osteogenic/vasculogenic engineered grafts to a vascular bundle in order to manufacture pedicled bone graft substitutes, which were then tested in an ectopic model of AVN. After 1 week in vivo, constructs with or without SVF cells were fully vascularized, but cell-based constructs displayed significantly (54%) higher blood vessel density. At 8 weeks, bone tissue was formed only in cell-seeded constructs. Implanted SVF cells contributed both to bone and blood vessel formation in vivo. Rat-derived osteoclasts and tissue remodeling (M2) macrophages were found predominantly in SVF-seeded constructs. We successfully engineered pedicled bone graft substitutes by combining a vascular bundle with SVF seeding. In the AVN model, the vessels arising from the central pedicle efficiently revitalized the necrotic outer shell, while the SVF cells were essential to attract/polarize M2 macrophages and to induce bone and vessel formation in the inner core. Longer implantation times are now required to investigate the dynamics of osteoconduction into the outer layer of the construct, which would bear relevance for the treatment of AVN.

Session No.: P2-042

The Application of Injectable Allogeneic Osteogenic Micro-tissue in Repair of Large Segmental Bone Defect

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[Abstract] PURPOSE: To investigate the feasibility of the application of injectable bone tissue engineering based on osteogenic micro-tissue in the large segmental bone defect. METHOD: We first harvested a dense osteogenisis cell sheet from the fetal rabbit bone marrow mesenchymal stem cells, which were induced in osteogenisis induced culture for 4 weeks. Then, we cut the cell sheet into fragments, and mixed the fragments with 1.5% sodium alginate solution, adding CaCl2 solution to prepare the osteogenic micro-tissue. After that, the osteogenic micro-tissue was injected subcutaneously to test its ectopic osteogenisis potential. Finally, we injected the osteogenic micro-tissue into the scaffolds, which were used for the repair of the critical bone defects, as to demonstrate its in situ osteogenisis potential. The calcium alginate gel without the cell fragments as the control group. After the X-ray examination, specimens were harvested in the fourth week. Computed tomography scanning and histological examinations were performed. The results were analyzed by paired Student’s t-test with SPSS 13.0 soft package. RESULTS: The fetal rabbit BMSCs showed good ability of proliferation and differentiation. The osteogenisis cell sheets showed a good characteristic of osteogenic differentiation. Computed tomography scanning and histological examinations confirmed new bone formation in the ectopic and in situ of the osteogenic micro-tissue group. CONCLUSION: The study indicates that osteogenic micro-tissue has a good osteogenisis potential, and can be used in the repair of segmental bone defect. It will simplify the process of bone tissue engineering, and shorten the waiting time before the BTE (bone tissue engineering) treatment.

Session No.: P2-043

Characterization of Decellularized Scaffold Derived from Porcine Meniscus for Tissue Engineering Applications

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Meniscus is a fundamental fibrocartilaginous organ distributing stress and transmitting load to reinforce the stability of knee joints. Trauma can injure the meniscus, while no specific therapy exists at present. The removal of damaged meniscus can impair normal knee function as well as predisposes patients to osteoarthritis. The aim of this study was to prepare decellularized meniscus scaffold using a 1% (w/w) sodium dodecyl sulfate solution and sufficient rinsing steps. Complete cell removal was verified through hematoxylin and eosin staining and DNA content assay. Both macro and microstructure were not affected by the decellularization process. Compared to the intact meniscus, the decellularized meniscus had accordant tension properties but with a decline in compression properties. This occurred because the collagen fiber was not damaged but glycosaminoglycans was significantly lost through the decellularization process, which was confirmed by biochemical assay and histology staining. The cytotoxicity of decellularized scaffolds was determined by contact and extract assay in vitro. There was no toxicity on L929 murine fibroblasts and porcine chondrocytes. In addition, not only could porcine chondrocytes adhere and proliferate on the scaffold surface, but some cells can infiltrate into the scaffolds. Although the number of infiltrated cells was precious few, it showed the potential of this decellularized meniscus to be the scaffolds in tissue engineering.

Session No.: P2-044

Comparison of Carbodiimide and Glutaraldehyde to Crosslink Tissue Engineering Scaffolds Fabricated by Decellularized Porcine Menisci

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Shattered decellularized meniscus was used to fabricating scaffolds to solve the problem that the pore size of decellularized meniscus is too small to let cells infiltrate into. Different concentration of 1-ethyl-3-3-dimethyl aminopropyl carbodiimide (EDAC) and glutaraldehyde (GTA) were used to crosslink the scaffold. GTA can bridge two collagen molecules with new chains, while EDAC can drag two collagen chains closer and form carbonyl groups between them. The change in molecular chains affected the micro structure of scaffolds. SEM (scanning electron microscope) photography showed that collagen fibers in high concentration GTA or EDAC groups had blurry edges. Scaffolds crosslinked by 1.2 M EDAC had significant smaller porosity and absorption than other groups for the fusing of collagen fibers decreased the porosity of scaffolds. Chemical crosslinking also improved the anti-degradation properties of scaffolds, 1.2 M EDAC or 2.5% GTA crosslinked scaffolds totally maintained their mass under the existing of collagen enzyme for 96h. Moreover, chemical crosslinking enhanced mechanical properties. The compression modulus of scaffolds crosslinked by 1.2 M EDAC or 1.0% GTA was three times than uncrosslinked ones. Tensile modulus was also improved more than 40 times after crosslinked by 1.2 M EDAC or 1.0% GTA. Cytotoxicity assay showed that scaffolds crosslinked by 1.0% and 2.5% GTA had toxicity to chondrocytes, while other groups showed no cytotoxicity. Chondrocytes were seeded into scaffolds for 7 and 14 days. SEM and live/dead staining demonstrated that cells proliferated and infiltrated within the scaffolds. Cell viability assay showed that cells in EDAC crosslinked scaffolds had higher cytoactivity than cells seeded in GTA crosslinked scaffolds. Overall, EDAC was a better crosslink agent in anti-degradation and cytotoxicity than GTA. Even though, the mechanical properties of crosslinked scaffolds is still inferior than intact meniscus, and further investigation should be focused on this aspect.

Session No.: P2-045

Study of the Regenerative Effects of Xenogeneic Transplantation in Knee Articular Cartilage Defects

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Objective: While allogeneic transplantation has attracted much attention in regenerative medicine, animal models approximating safety and efficacy are continued to be explored in translational research because cell sources and transplantation modalities are diverse. Currently, we are preparing for a clinical study using allogeneic chondrocyte sheets for articular cartilage regeneration. In the present study, we investigated the regenerative effects of human chondrocyte sheets for articular cartilage regeneration in a xenogeneic transplantation model using immune deficient rats.
Method: Articular chondrocytes and synoviocytes were obtained from patients (1 male, 1 female) undergoing total knee arthroplasty. They were co-cultured on temperature-responsive culture inserts for 2 weeks, and the chondrocyte sheets were then triple-layered and cultured for another week. Synoviocytes used as feeder cells were recovered and pelleted for transplantation. An osteochondral defect on the patellar groove of the femur was created on one knee of each F344/NJcl-rnu/rnu. Three groups (each n=6) were created as follows: group A (untreated control); group B (chondrocyte sheet transplantation); group C (synoviocyte transplantation). Rats were sacrificed at 4 weeks, and knee sections were evaluated histologically and scored using the International Cartilage Repair Society (ICRS) grading system.
Result: In group B, defects were filled with cartilage-like tissue that stained
for safranin O and type II collagen. In group C, defects were filled with fibrous tissue that stained for type I collagen. In groups B and C, parts of the tissue stained with anti-human vimentin antibody. The ICRS scores were as follows: group A (19.4±3.1), group B (26.9±5.7), and group C (21.5±1.9) with a significant difference between group A and B ($p<0.05$).
Conclusion: This model proved useful in evaluating the regenerative effects of human chondrocyte sheets. We plan to optimize allogeneic cell sheets by comparing these results with those from future transplantation of allogeneic cell sheets.

Session No.: P2-046

Decellularized Xenogenic Cartilage - Feasible Biocompatible Material for Vocal Fold Augmentation

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Objective The object of this study is to evaluate the efficacy of decellularized xenogenic cartilage as a long-lasting material for injection laryngoplasty and compare with other commercially available long-lasting material(Radiesse®, Artesense™) in the aspects of the foreign body reaction and local inflammation around injected substances. Material and Methods Porcine auricular cartilage was harvested under Institutional Animal Care and Use Committee(IACUC) approval. After freezing and grinding of harvested cartilage, we processed a decellularization protocol with 1% Triton X-100. Then we injected decellularized xenogenic cartilage into the subcutaneous layer of Sprague-Dawley rats. Results Giant cell infiltration around injected substances was significantly lower in porcine xenogenic cartilage injection group compared with Artesense™ and Radiesse® injection group. Neutrophil infiltrations were observed in xenogenic cartilage group and Artesense™ group compared with Radiesse® group at 1 month. However, at 3 months, neutrophil counts were decreased in xenogenic cartilage group and Artesense™ group, so the final neutrophil infiltration were very low in all groups. Conclusion Decellularized xenogenic porcine cartilage is quite feasible long-lasting material for injection laryngoplasty and has excellent advantages in the aspects of foreign body reaction compared with existing commercially available injection materials.

Session No.: P2-047

Lung Image Enhancement of Iodine Contrast Agent via Variant Delivery Route Under Computer Tomography

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Cancer is one of the leading causes of death in Taiwan. Lung cancer has the highest death rate among all cancers. Because early diagnosis rate of lung cancer is low; therefore, long-term survival rate of lung cancer is also quite low. So a method for diagnosing lung cancer in early stage is urgently needed. Nowadays, computed tomography (CT) is one of the non-invasive imaging tools commonly used to diagnose lung cancer. Iodine-based contrast agents are typically used to enhance the CT images contrast. In this study, there were two methods used to deliver iodine contrast agent (Iohexol) to evaluate the lung image quality via CT examination, that intravenous injection (IV) and inhalation were used to administer 500 mg/mL (50 μL) of Iohexol to mice. Micro-CT was used to observe how the Iohexol to influence the lung CT images of the mice at different time intervals, and blood samples were collected for biological analysis such as glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), blood urea nitrogen (BUN), and creatinine levels. Histological sections of organs (lung, kidney, liver, spleen) were evaluated for the possible damage of Iohexol delivered by different ways. The results showed that the pulmonary route of administration in which the Iohexol was delivered directly to the lungs caused clearer lung images and sustained images for up to 120 min. Iohexol delivered through intravenous injection (IV) remained in the body for a very short time and accumulated in the bladder only 5 min after administration. And also no differences of histological sections were observed in the experimental and normal organs between these two deliver methods. This study confirmed that inhalation delivery of iodine contrast agent (Iohexol) can enhance the contrast of lung images under CT examination and provide better and clearer lung images for early diagnosis.

Session No.: P2-048

Development of Catechol Conjugated and Chitosan Modified Double Concentric Calcium Phosphate Nanoparticles for Gene Delivery

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Calcium phosphate (CAP) based gene carrier preserve an attractive option for gene transfection due to their high biocompatibility and biodegradability property. Although there is great potential for the use of CAP in the development of gene delivery systems, the uncontrollable growth of CAP crystal and low protection of pDNA ability make it difficult to deliver genetic materials into cells. In this study, the transfection efficiency is optimized by varying the stoichiometry (Ca/P molar ratio) of the double concentric layers of calcium phosphate (DCP) and catechol-conjugated DCP with surface modification by chitosan (Chi). Our results indicate that the increase of Ca/P ratio (up to 700) will restrict the size of CAP crystal growth. Moreover, the degree of dispersion, stability and reproductivity can also be adjusted by repeated CAP precipitation steps. A bioadhesive molecule from marine mussels, 3,4-dihydroxy-L-phenylalanine (dp), is successfully used to bridge inorganic Ca2+ of DCP and organic Chi-functionalized DCP (Chi-dp-DCP). The positive surface charge of Chi-dp-DCP not only improves the binding affinity with plasmid DNA (pDNA) but also obtains better condensation efficacy when compared to DCP only or Chi-DCP group. The efficiency of pDNA protection could be considerably increased by adding another layer of Chi-dp on the DCP surface, resulting in successfully incorporating pDNA into the nanocarrier and preventing its degradation within the cell by lysosomes. It is suggested that Chi-dp-DCP nanocarrier has good DNase resistant effect, better biocompatibility, and high transfection efficiency. This stabilized CAP-based gene delivery platform could be considered a promising candidate as gene delivery carrier.

Session No.: P2-049

Dual Ionic Copolymer for Injection of Dna Vaccination by Multilayer Polymer Microneedle

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Delivery of DNA vaccines via microneedles (MNs) has more advantages over conventional intradermal injection. Here we report a novel dual ionic copolymer OSM-(PEG-PAEU) which delivers polyplex-based DNA vaccines using MN arrays. Fundamental characteristics of copolymer such as NMR, GPC, particle size, zeta potential, cytotoxicity are investigated. In addition, the fluorescence microscope and SEM images indicate that polyelectrolyte multilayers assemble (PMA) on MNs successfully. Moreover, we demonstrate the ability of release of PMA by pico green assay as well as transfection assay of functional polyplex released from PMA-MNs.

Session No.: P2-050

The Drug Delivery Behavior in Mixed Micelle of Various Composition of Atactic-PHB-mPEG and Isotactic-PHB-mPEG

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Amphiphilic block copolymers synthesized from biodegradable polymers have been explored in recent years. In an aqueous environment, they can self-assemble into polymeric micelles. Because of their unique features, such as small particle size, long-circulation time, and enhanced drug loading efficiency, micelles are widely used in drug delivery system. In this study, we synthesized two types of diblock copolymers. For the first type, the diblock copolymer contains atactic poly[(R,S)-3-hydroxybutyrate] (a-PHB) and methoxy poly(ethylene glycol) (mPEG) as the hydrophobic and hydrophilic block, respectively. This copolymer was synthesized via ring opening polymerization of -butyrolactone with mPEG as precursor. The second type diblock copolymer was synthesized from methoxy poly[(R)-3-hydroxybutyrate] (i-PHB) and mPEG with 1.6-hexamethylene diisocyanate (HMDI) as a coupling agent. These two types of copolymers showed significant difference in terms of their ability to form crystalline phase during micelle formation. The i-PHB-mPEG copolymer synthesized from nature PHB is semi-crystalline, while a-PHB-mPEG copolymer synthesized by the ring-opening polymerization is a totally amorphous. Therefore, the properties of the micelles can be tuned by mixing two types of copolymers in various ratios. The micelles with mixed copolymers were prepared by oil-in-water emulsion solvent evaporation method. The average diameter of the micelles is below 200 nm. From the observation of dynamic light scatting (DLS) and critical micelle concentration (CMC) experiments, it could be found that copolymers with the larger hydrophobic/hydrophilic ratio would form nanoparticles (NPs) in larger size and lower CMC, respectively. Furthermore, both higher loading capacity and slower in vitro release of CPT were observed for micelles of ROA-C, attributed to both improved drug-core compatibility and favorable amorphous core structure. The use of these two systems give us the ability to improve controlled drug release by tuning mixing ratio of crystalline/amorphous micelle, thereby being available to a variety of drug delivery applications.

Session No.: P2-051

The Development of Minoxidil Encapsulated Microneedles

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A novel drug deliver method which called microneedles (MNs) has been widely focused. Compared to traditional delivery systems such as dressing and in-situ injection, microneedles has higher efficiency of drug delivery than dressing and is less pain than in-situ injection. Microneedles can pierce through stratum corneum and has limited penetration depth to stimulate nerves, so it is painless. Base on the above advantages of MNs, we used carboxymethyl cellulose (CMC) which has been approved by Food and Drug Administration (FDA) to fabricate dissolvable microneedles with minoxidil encapsulated. Minoxidil is widely used to treat androgenic alopecia which is a common disease happening to male adult at the middle ages. With the decreasing age of alopecia patients in recent years, androgenic alopecia has attracted more attention. Minoxidil has been proven as having powerful effect on stimulating hair growing. The treatment was suggested to include a concentration of 5 wt% for men, and a concentration of 2 wt% for women. However, the current method of minoxidil drug delivery has low efficiency thus limited hair regrowth. To address the present problem, we design a dissolving CMC-MNs patch to encapsulate minioxidil. The mechanical strength of MNs will be enhanced to penetrate stratum corneum and the drug release of minioxidil through MNs will be tested. In this study, we expect to improve the drug uptake through skin and develop a potential commodity for androgenic alopecia patients. Keyword: dissolving microneedles, carboxymethyl cellulose, minoxidil, androgenic alopecia.

Session No.: P2-052

The Study of Hydrophobic Drug Encapsulated by Polypeptide Thermosensitive Hydrogel

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Tacrolimus (FK506) is an immunosuppressive agent which is usually applied to patients who received allotransplantation to reduce immune rejection. The strong hydrophobicity of FK506 makes it difficult to be applied in aqueous system. It has been studied to be encapsulated by micelles. However, the low rate of encapsulation in micelle is an obstacle for further application. Polypeptide thermosensitive hydrogel is considered as a great candidate for drug delivery because of several advantages, such as excellent biocompatibility and biodegradability, low concentration of critical gelation and sensitive response to the stimulation of temperature. In this study, we synthesized a thermo-responsive polypeptide hydrogel by copolymerizing poloxamer (PLX) and poly(L-alanine) with L-lysine segments at the both ends to form PLX-b-poly(L-alanine-lysine) (P-Lys-Ala-PLX) copolymers. Poly(L-alanine) is the hydrophobic chain of P-Lys-Ala-PLX copolymers which was designed to capture the hydrophobic agents. The result of synthesis was performed by 1H NMR and showed that P-Lys-Ala-PLX copolymers was successfully synthesized. P-Lys-Ala-PLX has a sol-gel behavior with low concentration of 3-7 wt% and has high degree of drug encapsulation. The experiment of drug release also showed that the FK506 released constantly over time. This study indicates that P-Lys-Ala-PLX is a high potential injectable material for biomedical application. Keywords: Polypeptide, thermosensitive hydrogel, tacrolimus, allotransplantation

Session No.: P2-053

A Novel Supramolecular Nanoparticle for Drug and Gene Delivery

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Over the past decades, significant efforts have been devoted to exploration on the use of nanoparticles in the fields of biology and medicine. Several different types of nanoparticles have successfully made their way into preclinical studies in animals, clinic trials in patients, or even successful commercial products used in routine clinical practice. Such as gold nanoshells, quantum dots and so on. The quantum dot can be tracked by UV while gold nanoparticle can be imaged by infrared radiation. However the toxicity of Quantum dot and Gold nanoparticle is still a problem for the really utilize of these nanoparticles. Carbon-based quantum dots, compared to traditional semiconductor quantum dots and organic dyes, photo luminescent carbon-based quantum dots are superior in terms of high aqueous solubility, robust chemical inertness, facile modification and high resistance to photo bleaching. The low toxicity and good biocompatibility of carbon-based quantum dots make them with potential applications in bio-imaging, biosensor and biomolecule drug delivery. So, we design a supramolecular system which is based on carbon dots supramolecular nanoparticles. This kind of nanoparticles was fabricated by using polyetherimide backbone which has high transmembrane efficiency. Then they were wrapped with polyethylene glycol and carbon dots by supramolecular interaction. In this way, our nanoparticles can bind hydrophobic drug and positive charged RNA. Then the supramolecular assembly will unfold and release the cargo in intracellularly with high efficiency.

Session No.: P2-054

Probing Cathepsin K Activity with a Selective Substrate of All Gold Forster Resonance Energy Transfer (AG-FRET) Is Associated with Arthritis

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Arthritis is one of the diseases common in the elderly, and patients in accordance with the conditions of daily activities will be different problems, so that patients in mental as well as physical in their daily lives are subject to considerable influence. Therefore, therapeutic intervention will benefit from the development of an effective and cheaper diagnostic technology for detecting and measuring progression in the early stages of arthritis disease. Cathepsin-K is one of the factors considered to be important in the chondrolytic processes that contribute to the degenerative changes in arthritis cartilage. We have developed a AG-FRET system (gold nanorods-peptide sequences-gold nanoclusters (GNRs-PSs-GNCs)) probes to detect Cathepsin K. The design reason is the GNCs can emit near infrared fluorescence wavelengths of 710-730 nm and GNRs can be used for quenching materials in “turn-on” fluorescent sensing systems. In addition, gold has chemically stability and bio-safety material and the AG-FRET incorporates a specific peptide substrate to native Cathepsin K can be quantified by spectrofluorometer and/or visualized in vivo imaging by in vivo imaging system (IVIS). These results include the synthesis and characterization of AG-FRET system. And then, the GNRs-PSs-GNCs probes were incubated with Cathepsin-K, the fluorescence recovered and the fluorescence intensity markedly increased in proportion to the amount of Cathepsin-K. We also used AG-FRET system to evaluate the synovial fluid from knee joints animal model for cartilage damage and inflammation.

Session No.: P2-055

Multifunctional Liposome Drug Delivery System Incorporated with Dual Probes of Magnetic Resonance Imaging and Fluorescence Imaging

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The iron oxide (Fe3O4) magnetic nanoparticles (MNPs) is a T2 contrast agent in magnetic resonance imaging (MRI). The other hand, the gold nanoclusters (GNCs) contains tens of atoms with subnanometer dimensions, possess superb red emitting fluorescence property which can avoid in vivo autofluorescent background, and own very low cytotoxicity. Therefore, the aim of this study is develop the multifunctional liposome which loaded with nanocomposites (Fe3O4@GNCs) comprising of MNPs of iron oxide and GNCs. First, the MNPs of iron oxide were synthesized by co-precipitation and their surfaces were modified amine groups by 3-Aminopropyltriethoxysilane (APTES). Second, the GNCs synthesis consists of reducing HAuCl4•3H2O by NaBH4 in the presence of lipoic acid (stabilizers) which, by adsorption on growing particles, ensures the control of the size and the stability of the colloid. And then, the dual image probes Fe3O4@GNCs were fabricated from MNPs of iron oxide conjugated with GNCs via amide bond formation. Finally, the liposomes nanocarriers enclosed these Fe3O4@GNCs in the inner phase by the reverse phase evaporation method. These results were characterized by particle size analyzer of dynamic light scattering, fluorescence spectrometer, X-ray diffractometer (XRD), transmission electron microscope (TEM), Fourier transform infrared spectrophotometer (FT-IR), superconducting quantum interference device (SQUID), MRI and in vivo imaging systems (IVIS) et al...

Session No.: P2-056

Preparation and Evaluation of Nanofiber Sheets by Electrospinning as a Sustained Drug Carrier

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The electrospinning technology has been extensively used as a method to make ultra-fine nanofiber sheets (NS) for biomedical engineering. NS has unique features, such as high surface area and ease of fabrication process. The unique features of NS lead to efficient vehicle in the required form sustained drug delivery. To advance a sustained dexamethasone (Dex) delivery, in the present study, we used the controllably biodegradable and excellent biocompatible poly(ε-caprolactone-co-L-lactide) (PCLA) copolymer. PCLA copolymer was synthesized and utilized to produce electrospun Dex-loaded NS using water-soluble Dex (Dex(s)) and water-insoluble Dex (Dex(b)). The Dex-NS were prepared by electrospinning, presented morphological similarities to natural extracellular matrix and interconnected fibrous structures, which were confirmed by field emission scanning electron microscope (SEM). The in vitro and in vivo degradation of Dex-NS was confirmed over a period of a few weeks by nuclear magnetic resonance (NMR) and gel permeation chromatography (GPC). The assessment of Dex(b) and Dex(s) release from Dex-NS showed an initial burst of Dex(b) at 1 day and, thereafter, the release maintained almost same amounts of Dex(b) for up to 4 weeks, indicating no and little releasing of Dex(b). In contrast, Dex(s)-NS showed a little initial burst of Dex(s) and the release pattern was first-order releasing profile from Dex-NS. In conclusion, Dex-NS showed the sustained Dex(s) releasing within implanted positions for extended action times, as well as to induce biodegradation of the NS over a defined treatment period.

Session No.: P2-057

Self-healable Supramolecular Nanogel Through Self-assembling of Specific Nucleobase Pairing as Drug Delivery System

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Hydrogels have been utilized extensively in the development of biomedical applications owing to the similarity in physicochemical properties with biological tissue. In order to deliver drugs with minimal invasion, injectable hydrogels have been highlighted during the past few decades. Unlike permanently cross-linked hydrogel, rapid self-healing of supramolecular hydrogels that are capable of autonomous healing upon applied stress is of great importance and potential applications. Since hydrogels with self-healing ability can be gelationed in situ and injected without fragmentation at the target site, it has become a desirable property making them attractive for tissue regeneration. In this study, Pluronic F127 (poly(ethylene glycol)-b-poly(propylene glycol)-b-poly(ethylene glycol)) will self-assemble in core-shell micelles with hydrophobic poly(propylene oxide) blocks as core and hydrophilic poly(ethylene oxide) blocks as corona. Then, these micelles will self-assemble through the hydrogen bond between adenine and thymine from functionalized Pluronic F127. When the temperature increases to 37 ℃, this smart hydrogel can undergo sol-gel transition and physically crosslink. Hydrogels which comprise discrete particles can also be called nanogels, and this nanostructured architecture can reduce the burst release of drugs so that it can improve the kinetic release profiles of encapsulated therapeutic agents. Due to the hierarchy of the nanogels, poorly water-soluble drugs can be incorporated into the poly(propylene oxide) hydrophobic core and hydrophilic drugs can be entrapped in the aqueous bulk of the nanogels. Therefore, this structure can endow hydrogels with different release profiles. Under applied shear stress, the nanogels will undergo reversible sol-gel transition and thus increase the release of entrapped drugs. We expect that the nanogels will exhibit self-healing behaviors and have controlled release property for carrying cargo with potential applications in the field of regenerative medicine.

Session No.: P2-058

Oxygen Clustering on Graphene Oxide Nanosheets for the Enhancement of Human Mesenchymal Stem Cell Differentiation

Jia-Wei Yang[1], Guan-Yu Chen[1], Sheng-Jen Cheng[1], Yu-Chih Shen[1], You-Rong Lin[1]

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Current method of stem cell differentiation is added growth factors or inducers in the cultural environment to maintain differentiation conditions. However, differentiation results in 3D scaffolds are often less than in 2D cell experiments that may be a complex internal structure or small pore size resulting in growth factors or inducers cannot fully permeate. Here we induce a phase transformation (oxygen clustering) in graphene oxide and provide a unique surface that allows acceleration of human mesenchymal stem cell differentiate into osteogenic lineage, and does not need additional additives. Overall, our work highlights a general route to improve functionalization of GO for stem cell applications.

Session No.: P2-059

Super-paramagnetic Scaffolds Modulate the Function of Macrophages Under the Static Magnetic Field

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Increasing evidence shows that magnetic fields and superparamagnetic responsive scaffolds can synergize to play unique roles in promoting bone regeneration for defects beyond critical size. Most of studies focused on the effects on osteoblast cells as well as stem cells, investigating cell differentiation in osteogenesis guided by the superparamagnetic scaffolds and magnetic field. Besides bone-related cells, immune cells especially macrophages have been recognized to play crucial role in regulating the wound healing process. In general, scaffolds made of biomaterials induce inflammatory reactions or foreign body reactions, which are essentially related with macrophages. We aim to get insights into macrophages response to the superparamagnetic responsive scaffolds with magnetic fields by identifying the phenotype changes of macrophages. Composite scaffolds made of poly lactide, hydroxyapatite and γ-Fe2O3 nanoparticles were fabricated using the electrospinning technique. Macrophages were seeded on scaffolds with or without magnetic fields. Experimental results indicated that macrophages cultured on super-paramagnetic scaffolds under magnetic fields tended to polarize into the immunomodulatory and tissue repair phenotype, showing that the increased expression of M1 marker, along with decreased expression of M2 marker. The secretion of angiogenic cytokines was upregulated while the secretion of inflammatory cytokines was decreased. In vitro angiogenesis assay demonstrated that the M2-like profile of macrophages induced by the scaffold under magnetic field not only enhanced endothelial cells to form networks but also promote the migration of pre-osteoblasts cells. This research showed that scaffolds combined with magnetic fields can inhibit the osteoclastogenesis of macrophages. This study also shows some possible mechanisms that may be correlated with the connection between macrophages and scaffolds with magnetic fields. In conclusion, the super-paramagnetic responsive scaffolds can synergize with magnetic fields to regulate the macrophage phenotype to enhance the regeneration and remodeling process, suggesting a promising strategy for bone repair and are worthy for further investigation.

Session No.: P2-060

The Critical-Size Calvarial Defect Repair Using Baculovirus-Engineered ASCs Co-Express BMP-2 and SDF1

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Bony defects in the craniofacial skeleton remain a challenging health concern. Our previous studies have shown that baculovirus (BV)-mediated gene therapy combined with adipose-derived mesenchymal stem cell (ASCs) to persistently express BMP-2 can improve the femoral bone defects. However, complete repair of the calvarial bone defects using the BV-mediated ASCs therapy remained difficult. Stromal cell-derived factor 1 (SDF-1) is a chemokine that can recruit mesenchymal stem cell (MSCs). Because of the inferior osteogenesis of ASCs, we hypothesized that implantation of ASCs persistently co-expressing SDF-1 and BMP-2 can recruit host MSCs and promote the osteogenic ability of ASCs, which synergistically promote calvarial bone healing. Therefore, we constructed a new Cre/loxP-based BV system encoding SDF-1 and BMP-2. Transduction of the rat ASCs with the new BV system conferred prolonged SDF-1 and BMP-2 co-expression, which recruited MSC in the transwell migration and synergistically promoted the osteogenic differentiation of transduced ASCs in vitro. Furthermore, implantation of the ASCs co-expressing BMP-2/SDF-1 into critical-size (6 mm in diameter) calvarial bone defects in SD rat accelerated the bone healing, filling ≈70% of bone volume with native calvaria-like flat bone in 12 weeks. Altogether, this study confirmed that BV-engineered ASCs co-expressing BMP-2/SDF-1 could synergistically stimulate the ASCs osteogenesis in vitro and improve the calvarial bone healing in vivo.

Session No.: P2-061

Accelerated Bone Regeneration Using Biodegradable and Transplantable Nanopatterned Patch

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Naturally bone healing process could take several weeks, months or even years depending on the injury size. In terms of bone healing speed, many researchers has been used various growth factor delivered with implantable biomaterials to both accelerate and ensure healing of bone fracture. However, there may have occurrence with side effects such as nerve pain, infection and ectopic bone formation. As an alternative method, we focused on the biophysical guidance, which was provided similar topographical cues to cellular environment, to recruit host cells for bone defect healing. In this study, we hypothesized that biomimetic nanotopographical features have enhanced cell recruitment, migration and differentiation from intact cells without additional stimulation. We designed a biodegradable and transplantable poly (lactic-co-glycolic acid) nanopatterned patch (NP) which was mimicked highly aligned extracellular structure in bone tissue using simple solvent casting and capillary force lithography. We confirmed that micropatterned patch and NP regulated osteoblast behavior according to orientation of pattern in osteoblast migration. These aligned osteoblasts might contribute to in vitro osteogenic activity such as alkaline phosphate activity, Von kossa staining and calcium contents compared to the flat patch (FP). To demonstrate bone defect healing by guidance of NP in vivo, we implanted whole and bridge patches on critical size defect of mouse calvarial (ø 4 mm) and analyzed to use microcomputed tomography and histology. Only NP treated group had faster new bone formation and compact bone regeneration at defect area compared to FP at 4 and 8 weeks. Especially, the bridge NP guided to be regenerated new bone formation along with the nanopattern. The NP with biophysical guidance should be suitable utilized for tissue regeneration by accelerated intact cell migration.

Session No.: P2-062

The Influence of Wollastonite and Macro-porous Fibrin Biphasic Scaffold on Osteochondral Defect Regeneration

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osteochondral defect refers to the damage of cartilage as well as subchondral bone. Cartilage tissue engineering focusing on the regeneration of cartilage regardless of the subchondral bone always leads to partial regeneration of the damage, resulting in poor mechanical and physiological properties. Therefore, osteochondral defect regeneration of both cartilage and subchondral bone is of great importance. A proper scaffold suitable for in situ inductive regeneration of both tissues is of urgent need. Wollastonite and macro-porous fibrin biphasic scaffolds loaded with rabbit bone marrow mesenchymal stem cells were fabricated and were used to induce osteochondral regeneration. The wollastonite scaffolds contain 8% MgSiO3, of which silicon ions can induce bone regeneration and magnesium is essential for skeletal metabolism, while bioactive macro-porous fibrin scaffold can induce cartilage regeneration. These two scaffolds were combined with fibrin gel. We assume the biphasic scaffolds are promising for the osteochondral defect regeneration. In vivo experiment was conducted by implantation of the wollastonite and macro-porous fibrin biphasic composite scaffold into full thickness osteochondral defects (4 mm in diameter and 4 mm in depth with bone marrow blood effusion) of New Zealand rabbit for 12 and 18 w. 12 w after implantation, the gross view confirmed the cartilage was regenerated from the edge into the center, and through the micro CT we can see the bone regeneration effect. Keywords: cartilage regeneration; composite scaffold; osteochondral; fibrin Acknowledgements This study is financially supported by the Natural Science Foundation of China (21434006, 21374097). References: [1] Goudouri O.M.,Vogel C.,Grunewald A., et al. J Biomater Appl, (2016),30: (6) 740-749 [2] Goudouri O.-M.,Kontonasaki E.,Chrissafis K., et al. Ceram Int, (2014),40: (10) 16287-16298 [3] Guo H.,Wei J.,Song W., et al. Int J Nanomedicine, (2012),7: 3613-3624

Session No.: P3-063

Microenvironmental Stimuli to Differentiate Adipose-derived Stem Cells for Injury Prevention in Nervous System

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Neonatal hypoxic-ischemic (HI) injury disrupts the neurovascular architecture and leads to life-long functional deficits. The devastating outcome can be ameliorated by preserving the endothelial or neural structures, but the source of therapeutic cells was limited. ASCs can differentiate into endothelial lineage cells (ELCs) using combination of biochemical and mechanical stimulations, or induce toward neuronal lineage cells (NLCs) by forming neurosphere on chitosan-coated surface. We tested the beneficial effect of combine NLCs with ELCs (E+N) to protect both neural and endothelial network in neonatal HI brain injured rats by transplanting cells via percutaneous injection. The E+N combination showed an even more significantly decrease of brain infarction and apoptotic area than ELCs transplantation. By observing the neurovascular architectures, we found the transplanted ELCs and NLCs can homing to the injured brain for specific vascular and neural structures as well as the boost-up of endogenous angiogenesis and neurogenesis. In addition, the combine treatment of E+N showed better recovery of memory for long-term cognitive function. To reveal the cell-cell interactions between ELCs and NLCs, we used Boyden chamber to assess the in vitro cell mobility and revealed an interesting synergetic increase of transmigration when combining ELCs and NLCs under hypoxic microenvironment. The interaction signal is involved with NRP1 signaling in ELCs and with the C-X-C chemokine receptor 4 (CXCR4) and fibroblast growth factor receptor 1 (FGFR1) signals in NLCs. Blockage of specific signals in either ELCs or NLCs diminished the beneficial effects of cell migration, homing, and protection of neurovascular structures in the E+N combined treatment. In summary, the microenvironmental cues provide induction factors for ASCs to differentiate into endothelial and neural lineages. The synergistic effect of combine ELCs and NLCs improved both structural and functional recovery for neonatal HI brain injury.

Session No.: P3-064

The Critical Role of Glucose in the Post-implantation Microenvironment for Ensuring Mesenchymal Stem Cells Survival.

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Mesenchymal stem cells (MSC) hold considerable promise in regenerative medicine. However, exogenously administered MSCs, when loaded into scaffolds, exhibited poor survival. A possible explanation for this limited cell survival is that, upon implantation, MSCs encounter a considerable bioenergetic challenge to ensure their homeostasis and fuel the regenerative response. The complexity of the in vivo environment makes it difficult to decipher the mechanisms by which Human MSCs (hMSCs) align their bioenergetic needs with available energy resources post-implantation. To investigate these aspects, an in vitro model that reflected the post-implantation environment was developed and the complete metabolic requirements of hMSCs was explored. To this aim, we determined, for the first time, that 0.1% oxygen level best reflected the in vivo situation. hMSCs exposed in vitro to 0,1% (but not to 1 or 5%) oxygen expressed the same hallmarks of ischemia than hMSCs located in the tissue constructs post-implantation. Under this condition, hMSCs had virtually no internal stock of ATPs and a limited one of glucose. Energy was provided to hMSCs through glycolysis and oxidative phosphorylation was downregulated. Moreover, serine and glutamine supply did not improve hMSCs survival suggesting an inactivation of serinolysis and glutaminolysis under near-anoxia. Most importantly, glycolysis was the exclusive energy-providing pathway for ensuring hMSCs survival in vivo because its inhibition induced a rapid decrease in ATP level, which was fatal to hMSCs in the short-run post-implantation. Last but not least, the critical role of glucose in the post-implantation microenvironment was further confirmed by ectopically implanting luciferase-labelled hMSCs constructs supplemented with glucose. Cell constructs loaded with glucose exhibited four- to fivefold higher hMSC viability and were more vascularized compared to those implanted without glucose at day 14. Given the wide interest in hMSCs for regenerative medicine, this new knowledge may be applied to engineer cell constructs that enhance hMSCs survival.

Session No.: P3-065

Role of Fibroblast Growth Factor 9 for Neurosphere Formation in Adipose-derived Stem Cells

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Cell-based therapy show promising results to increase nerve regeneration after injury. Adipose-derived stem cells (ASCs) have several advantages to be an ideal cell source for stem cell therapy. Our lab demonstrated the possibility of induce neurosphere by culturing ASCs on chitosan-coated dish. The fibroblast growth factor 9 (FGF9) was reported can alter the proliferation and cell-fate decision by binding to different FGF receptors (FGFRs) in stem cells. However, how FGF9 affects ASCs during the sphere formation is still unknown. In this project, we aim to investigate the role of FGF9 during neurosphere formation of ASCs. The ASCs was induced to form neurosphere by seeding on chitosan-coated dish and confirmed protein expressions in neural lineage. The gene expressions of FGF9 and FGFRs were also influenced during neurosphere formation. Second, we treated ASCs with different dosages of FGF9 peptide during sphere formation to study the role of FGF9 in neural differentiation and proliferation. By increasing the concentration of FGF9 peptide, neurosphere showed decrease of sphere diameter and expressed increase of nestin, GFAP, and S100 gene. Therefore, we investigated phosphorylation of ERK, JNK, and Akt expression during sphere formation by western blot with or without FGF9 treatment. During sphere formation, the signaling of p-ERK and p-JNK were decreased on day 3 and p-Akt was decreased after 1 day. The addition of FGF9 cause a slight increase of p-Akt at 6 hrs. The finding of current study may show the possibility of differentiating ASCs into desired neural lineage by modulating the level of FGF9 during sphere formation.

Session No.: P3-066

Scaffolds and Scaffold-free Cell Sheets for Repairing Articular Cartilage Defects

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Currently, arthroscopic lavage and debridement and microfracture are the main surgical options for repairing of articular cartilage defects in the mainland of China. Tissue engineering and regenerative medicine has caused a revolution in present and future trends of medicine and surgery. From a few years ago to now, there are two novel methods to be focused on to repair articular cartilage defects. The first method is cell-free matrix-induced chondrogenesis using acellular extracellular matrix scaffolds and the second is scaffold-free cell sheets implantation technique. The acellular extracellular matrix scaffolds were derived from animal tissues by decellularization and inactivation of virus and used to augment microfracture. The scaffold-free cell sheets had been prepared by chondrocytes, bone marrow mesenchymal stem cells, adipose-derived mesenchymal stem cells and umbilical cord mesenchymal stem cells through our multilayer cell sheets culture technique. Animal studies show that both methods are safe and effective for articular cartilage defects repair. Now, the cell sheets technique have been applied to 10 patents following by 2 years and all the patents have normal quality of life. Meanwhile, a multicenter, randomized controlled clinical study is recruiting to evaluate of safety and efficacy of the acellular extracellular matrix scaffolds for augmenting microfracture.

Session No.: P3-067

Assessment of the Difference of Cell Growth Rate 1: the Individual Differences at the Manufacturing Using Autologous Cells Under the Cancer Immunotherapy in Japanese Clinical

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Objective: There are many clinical applications using cell-based products under the Medical Practitioners Act and the Medical Care Act in Japan, and almost of them are autologous cell therapies. The quality control of these products is relatively difficult because their raw materials are derived from autologous cells of the patients. A raw material has a personal specific activity depend on the condition of donor patient. If the efficacy of a treatment modality was evaluated, individual differences containing in the products would be one of important issues. Therefore, we evaluated fluctuations due to raw materials in the cell expansions of peripheral blood mononuclear cells (PBMCs), which were accumulated by manufacturing for immunotherapy at the Biotherapy Institute of Japan (BIJ).

Methods: PBMCs as a raw material was isolated from 50 mL of peripheral blood, which was collected from a patient or a healthy donor, and the expansion using a natural killer cell expansion kit (BINKIT® , BIJ, Japan) was carried out. All the culture periods extracted was 21 days. After the expansion period, the total number of cells was counted and evaluated.

Results: Recent sixteen cases were picked up in a preliminary survey. The total cell number in most cases has reached more than 2 x 109 cells as the lower limit, which have been expected, but two cases were deviant. The average numbers of cells were around 4 x 109, and the cell growth rate of patients was close to that of healthy donors on an average. The details of individual scattering and more data will be described on the site.

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Session No.: P3-068

Preclinical Safety and Stability of hBM-MSCs Manufactured in the GMP Facility of University Hospital

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Prior to clinical research, cell therapy products, manufactured under GMP control, must prove preclinical safety as well as their stability. Mesenchymal stem cell (MSC) is one of the most promising cell types in the field of cell therapy, thanks to their extensive potentials. We have obtained human bone marrow from healthy donors after IRB approval and established GMP-grade MSCs (Catholic MASTER cells) by isolating plastic-adherent cells and expanding cell number through passaging, with proper quality control in the institutional GMP facility. We established extensive quality tests as in-process control and finally to meet lot release criteria. Final products were tested in their stability to determine storage condition and expiry date and the result showed that Catholic MASTER cells are stable at least for 48 hours after manufacturing. Preclinical safety tests were performed under Good Laboratory Practice. Catholic MASTER cells were administered into nude mice intravenously either at single dose or at multiple doses to evaluate their toxicity to find approximate lethal dose (ALD) or No-observed-adverse-effect level (NOAEL), respectively. The cells were also tested for tumorigenecity by injecting the cells into nude mice subcutaneously or intravenously. Biodistribution was also evaluated for 8 weeks after intravenous injection into the animals. ALD in single dose test was found to be higher than 2 million cells per animal (or 1x10^8/kg) and NOAEL in repetitive dose test was found to be 1 million cells per animal (or 5x10^7/kg). Tumorigenecity test verified that Catholic MASTER cells have no tumorigenic potential. Biodistribution test showed that the MSCs injected into nude mice intravenously could be detected for less than 4 days in some organs. Recently, a phase I investigational clinical trial using 'Catholic MASTER Cells' was approved by KFDA after intensive GMP audit.

Session No.: P3-069

Image-based Real-time Monitoring Method for Evaluating Undifferentiation Status of Induced Pluripotent Stem Cells Culture

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Induced pluripotent stem cells (iPSCs) are becoming one of the ideal cellular resources in regenerative medicine and drug discovery. To widely distribute iPSCs and promise their stable results for further applications, mass production of such stem cells is becoming strongly required. In such cell production, the cellular quality is considered to be assured by the concept of "quality by design (QbD)", which aim to assess and evaluate every steps of production. However, the conventional cell evaluation techniques in molecular biology had been found to be difficult in such QbD-based process design, because most of the assays are invasive, which is not able to test the actual product intactly. The morphology of iPSCs has been long considered to be one of the important information that can judge their undifferentiation status in everyday maintenance. We here repot the use of colony tracking technology based on soft-matching together combined with the informatics algorithm to systemize the manual morphology observation by experts by computational algorithms. By analyzing the proliferation profile from single colony tracking data, we show that our evaluation method can quantitatively evaluate their production efficiency continuously during their culture. Especially, our method was able to detect colonies that lost the lectin marker expression. We analyzed more than 2000 iPSC colonies, and show that "irregular morphology" can be defined by quantitatively measured morphological parameter combinations. Our results show that non-invasive image-based morphology analysis enables real-time quality record, monitoring, and prediction for assiting QbD-based iPSC production process design.

Session No.: P3-070

Assessment of the Difference of Cell Growth Rate 2: the Influence of the Storage Period After the Collection of Blood for Immune Cell Manufacturing in Japanese Clinical

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Objective: There are many clinical applications using cell-based products under the Medical Practitioners Act and the Medical Care Act in Japan, and almost of them are autologous cell therapies. A raw material has an individual specific activity depend on the condition of donor patient. If the efficacy of a treatment modality was evaluated, the conditional differences containing in the products would be one of important issues. Therefore, we evaluated the influences due to raw materials in the cell expansions of peripheral blood mononuclear cells (PBMCs), which were accumulated by manufacturing for immunotherapy at the Biotherapy Institute of Japan (BIJ). In this study, the influence of the storage period containing transit time after the collection of blood as a raw material was focused.

Methods: PBMCs as a raw material was isolated from 50 mL of peripheral blood, and the expansion using a natural killer cell expansion kit (BINKIT® , BIJ, Japan) was carried out. The peripheral blood collections were carried out at Tokyo or Fukushima in Japan. PBMC isolation was started in same day when the blood was collected at Tokyo, and the isolation was treated the next day (after 24 hours) when collected at Fukushima. All the culture periods extracted was 20±2 days.

Results: Recent 37 cases were picked up in a preliminary survey. The average number of cells collected was 3.6 x 109 when collected at Tokyo, and was 3.0 x 109 when collected at Fukushima. Both the growth rates of cells were close.

Session No.: P3-071

Development of Human Epidermis Skin Equivalent Epitri for in Vitro Skin Irritation Test

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Irritation and corrosion evaluation of human pharmaceutical and cosmetic products for topical use traditionally has relied on animal experiments. However, the trend has changed to in vitro testing due to the 3R (replacement, reduction, and refinement) requirements resulted from the recent animal testing ban implemented by REACH and new cosmetic regulation started from EU. The animals testing ban includes prohibitions to test cosmetic products and ingredients on animals (testing ban), as well as to market these products and ingredients in the European Union (marketing ban). We have established an efficient protocol for expansion and differentiation of primary human keratinocytes and developed the human skin epidermis equivalent named EPiTRI. Using our proprietary induction protocol, a multi-layered epidermis composed of stratified stratum corneum, granulosum, stratum spinousum and basal layer were formed after 14 days in air-lift culture and resembled in vivo skin epidermis structure. The values of the transepidermal electrical resistance (TEER) determined by the Millipore-ERS Volt/ohm-meter are correlated to the tightness of cell layers. The TEER values of EPiTRI were assessed in every other day during air-lift cultivation. The results showed that during maturation of EPiTRI, the TEER values were increased from approximately 0 to 12 kΩ. The highest TEER value was observed at 11 days after air-lift culture in EPiTRI. Moreover, immunocytochemical staining showed that loricrin, cytokeratin 10 and cytokeratin 14 were expressed in the granulosum, spinousum and basal layer respectively, indicating that the reconstructed human epidermis skin equivalent has the correct cellular organization. The skin equivalent also exhibits reproducible barrier function and qualified the skin irritation test conducted in accordance with OECD439. The skin epidermis equivalent EPiTRI can provide a good model to evaluate cosmetic products and ingredients for skin irritation assessments.

Session No.: P3-072

3D Printed Zirconium/Silicate Hybrid Ceramic Scaffold with Balanced Mechanical Stability and Cell Compatibility

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The additive manufacturing of bone scaffold for the clinical applications is an emerging field in orthopedics. Therefore, the scaffold should possess the balanced mechanical characters and bioactivities to ensure the supporting strength for stress bearing and the penetration of cells or tissues. In order to achieve the demands in tissue engineering, two kinds of ceramic were composed in this study, one was the bioactive ceramic, silica (SiO2), and the other was zirconia (ZrO2), could provide high resistance to crack propagation. Different formulas of ceramic composite were employed in specimen manufacturing by selective laser gelling method to evaluate the biomedical and mechanical properties. According to the results, the proportion composed with 50% SiO2 and 50% ZrO2 (S5Z5) exhibited the balanced mechanical properties and cell compatibility. After sintering at 1300 ℃, the compressive strength and bending strength of S5Z5 were 70.76 Mpa and 45.57 Mpa, respectively. Furthermore, the S5Z5 ceramic composite presented no cytotoxicity and acceptable cell affinity. The clinical application of S5Z5 ceramic composite would be further approved by animal experiments in subsequent study.

Session No.: P3-073

Fabricating a Novel Integrated Valved Scaffolds for Bioengineering Applications via TIPS Based on 3D Printing and CFD Analysis

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A variety of congenital heart disease (CHD) with severe right ventricular outflow tract (RVOT) obstruction or interruption requires surgical reconstruction mainly valved conduit replacement. Widespread efforts have been made with homologous valved conduit and xenologous valved conduit, which showed failure in recent clinical trial due to severe shortage of donors and have no growth potential, particularly in young patients. An suitable valve scaffold prosthesis is necessary under these conditions. Combined the latest technology with the development of biomaterials in recent years, we fabricated a novel integration valved conduit to solve these problems, in which consideration must be given to both the design requirements and the behavior of physiological, along with good biocompatibility, meet the characteristics of hemodynamic, for basic research and clinical application. We produced a special mold using computer-aided design (CAD) combined with the 3D printing technology, using the thermally induced phase separation (TIPS) to prepared a integration valved conduit with Poly(L-lactic acid) / Poly(L-lactide-co- ε-caprolactone) (PLLA / PLCL). Computational fluid dynamics (CFD) analysis and mechanical analysis has been completed to optimization the integration valved conduit. In addition, computational hemodynamic simulation analysis was applied to test the function of valved scaffold in the pulsating flow field. In this study, we developed and implemented a TIPS-based strategy combining 3D printing mold with biodegradable materials to rapidly fabricated a novel integrated valved scaffold exhibiting favorable mechanical properties and satisfied functional performance. Moreover, excellent biocompatibility and degradation property for regeneration of the biomaterials has been proved in vivo. Our research supported that this original strategy was an efficient process for fabricating integrated valved scaffolds with potential value in clinical application of complex CHD. Furthermore, CAD-based 3D printing technology and CFD analysis for effective design and optimization of the valved scaffolds will benefit to the personalized treatment and clinical transformation in the future.

Session No.: P3-074

Micropatterning of Neuro-2A Cells on Modified Chitosan Substrates

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In order to make use of nature-driven biomaterial for nerve tissue engineering, we develop physically modified chitosan substrates to pattern Neuro-2a cells. To prepare chitosan substrates with micro/nano-scaled architectures, we demonstrated an easy-to-handle approach that was reached through biofabrication, which is a combination of photolithography, inductively coupled plasma reactive ion etching (ICP-RIE), Ag nanoparticle-assisted etching, and solution casting. Our experiment showed that Neuro-2a cells preferred to adhere to flat chitosan surfaces rather than nanostructured chitosan surfaces as evidenced by greater spreading and differentiation, pointing out that surface topography can be used as a strategy for patterning neural cells. Based on this experience, we developed chitosan substrates with six types of micropatterns including squared micropatterns, reverse-squared micropatterns, single-cell micropatterns, line micropatterns, network micropatterns and polarity micropatterns, which allowed us to re-arrange Neuro-2a cell colonies at desired positions. Each micropatterns has different outcomes for pattering Neuro-2a cells. In particular, we found that the polarity micropattern provided the most efficient surface pattern for guiding Neuro-2a cells on a chitosan substrate. The cellular polarity of a Neuro-2a cell spreading correlated to a diamond-like pattern. In addition, neurite outgrowth was induced from the corners toward the channels of the structures. We believe that the study has the potential to provide greater insight into the development of nerve tissue engineering, including nerve repair scaffolds, and in vitro platforms for electrophysiological stimulation or neurotransmitter screening.

Session No.: P3-075

Possibility of Applying Photo-Acoustic Method in Term

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We developed newly designed a photoacoustic measurement system. Photoacoustic imaging based on optical absorption and ultrasonic detection is actively being studied for biomedical applications. Photoacoustic imaging, also called optoacoustic imaging, provides cross-sectional or three-dimensional images, noninvasively. Because acoustic waves scatter much less than optical waves in tissue, Photoacoustic imaging has the advantage of imaging that can penetrate deeply. Another advantage is spectroscopic-based specificity using endogenous and exogenous optical contrasts. This imaging technique is characterized by the scalability of its spatial resolution and depth penetration across both the optical and ultrasonic dimensions. We try to apply this photoacoustic imaging technology to validate tissue-engineered tissue. We will discuss the applicability of the photoacoustic imaging for validation of tissue-engineered tissue.

Session No.: P3-076

The Penetrated Delivery of Drug and Energy to Tumors by Bio-inspired Nano-materials

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A magneto-responsive energy/drug carrier that enhances deep tumor penetration with a porous nano-composite is constructed by using a tumor-targeted lactoferrin (Lf) bio-gate as a cap on mesoporous iron oxide nanoparticles (MIONs). With a large payload of a gas-generated molecule, perfluorohexane (PFH), and a hydrophobic anti-cancer drug, paclitaxel (PTX), Lf-MIONs can simultaneously perform bursting gas generation and on-demand drug release upon high-frequency magnetic field (MF) exposure. Biocompatible PFH was chosen and encapsulated in MIONs due to its favorable phase transition temperature (56 ℃) and its hydrophobicity. After a short-duration MF treatment induces heat generation, the local pressure increase via the gasifying of the PFH embedded in MION can substantially rupture the three-dimensional tumor spheroids in vitro as well as enhance drug and carrier penetration. As the MF treatment duration increases, Lf-MIONs entering the tumor spheroids provide an intense heat and burst-like drug release, leading to superior drug delivery and deep tumor thermo-chemo-therapy. With their high efficiency for targeting tumors, Lf-MIONs/PTX-PFH suppressed subcutaneous tumors in 16 days after a single MF exposure. This work presents the first study of using MF-induced PFH gasification as a deep tumor-penetrating agent for drug delivery.

Session No.: P3-077

A Photodynamic Nanoparticle for Inhibition of ROS Generation in Stimulated Macrophages

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We prepare a photodynamic selenium nanoparticle (SeNPs) to target activated macrophage using a layer-by-layer assembly method. The SeNPs were coated with rose bengal-chitosan-glutathione conjugate (RB-CS-GSH) and hyaluronic acid-folic acid conjugate (HA-FA) conjugates. Surface-modification of the SeNPs with the HA-FA conjugate endowed the SeNPs with CD44 and folate receptor β (FR-β) mediated macrophage targeting. Cellular uptake of the RB-CS-GSH and HA-FA igand-modified SeNPs by lipopolysaccharide (LPS)-stimulated macrophages were enhanced due to the receptor-mediated endocytosis. Photodynamic treatment of the macrophages with the SeNPs can efficiently reduce ROS and nitric oxide (NO) production by the cells. These observations reveal that the photodynamic SeNPs might be excellent in treating macrophage-related chronic inflammatory diseases with minimized side effects. Keywords: chitosan, Se nanoparticles, photodynamic, macrophage

Session No.: P3-078

Design and Preparation of Antibody-immobilized Gelatin Nanoparticles Incorporating a Molecular Beacon to Visualize the Biological Function of Macrophages

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The objective of this study is to design and prepare a nanoparticle-based fluorescent probe to visualize the biological function of macrophages through the intracellular detection of an inflammation-related microRNA. The gelatin coacervation was formed by adding the acetone to the gelatin aqueous solution, followed by the chemical crosslinking with glutaraldehyde (GA) to obtain gelatin nanoparticles. Next, an antibody with an affinity for the surface ligand of macrophage was immobilized on the gelatin nanoparticles through the reductive amination reaction between the amine groups of gelatin chain and the sugar chain of antibody. A nucleic acid-based molecular beacon (MB) for a pro-inflammatory microRNA (miR-155) was designed. The MB shows the fluorescent recovery from the quenched state through the structural change of MB by the interaction with miR-155. The MB for miR-155 was mixed with a peptide derived from a trans-activator of transcription (TAT) protein. The MB-TAT complex was added to antibody-immobilized gelatin nanoparticles followed by the purification to obtain the antibody-immobilized gelatin nanoparticles incorporating MB (MB-gelatin NP). RAW264.7 cells of a macrophage-like cell line were differentiated into pro-inflammatory or anti-inflammatory macrophages by culturing in the media containing lipopolysaccharide and interferon-gamma or interleukin-4, respectively. It is reported that the expression level of miR-155 in pro-inflammatory macrophages is significantly higher than that in anti-inflammatory macrophages. Each pro-inflammatory or anti-inflammatory macrophages were cultured with the MB-gelatin NP. Then, the microscopic observation and detection of cellular fluorescence were performed by a fluorescence microscope and flow cytometer. The fluorescent intensity of pro-inflammatory macrophages cultured with the MB-gelatin NP was stronger than that of anti-inflammatory macrophages. It is conceivable that the MB-gelatin NP was internalized into the pro-inflammatory macrophages and the MB released from the nanoparticle in the cytosol was interacted with the miR-155, resulting in the strong emission of fluorescence through the structural change of MB.

Session No.: P3-079

Size-changeable Graphene Quantum Dot Nano-aircrafts for Penetrated Drug Delivery and Photothermal Therapy

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Delivery of therapeutic cargoes within carriers that effectively accumulate at tumor promotes mitigating side effects and improving the tumor therapeutic efficacy demanded in personalized medicine. Due to the tumor heterogeneity, however, these carriers which usually display low tumor accumulation and are piled up at the tumor's periphery near the blood vessels must address the issues in accumulation, penetration and transport sufficient cargoes to the deep tumor for effective therapy. Here, a size-changeable graphene quantum dot (GQD) nano-aircraft that doubles the efficiency of tumor accumulation and possesses high cargoes payload is developed to penetrate and sequentially release drugs into deep tumors through near-infrared irradiation. The nanotheranostic composed of ultrasmall GQD (5 nm) functionalized by amphiphilic pH-sensitive N-acetyl histidine-conjugated D-α-tocopherol polyethylene glycol 1000 succinate (HTPGS) that changes its size and aggregates at tumor through pH-sensitive surface exhibits a double increase in accumulation than the carriers without modification. Furthermore, this nanotheranostic carries a large payload of an anticancer drug, doxorubicin (DOX), on GQD and possesses long half-life in circulation due to polyethylene glycol (PEG) of HTPGS. A size conversion of nanotheranostics at the tumor site was actuated by NIR irradiation which disassembles 450 nm of nanotheranostic clusters into 5 nm of DOX/GQD like bombs-loaded jets, facilitating the penetration into the deep tumor tissue far from blood vessels, achieved by atomic-thin structures of GQD and hyperthermia. Followed by sequentially releasing DOX near the nucleus, GQD nanotheranostic integrates a penetrated photothermal-chemo therapy. Such size-changeable nanotheranostic integrated combination therapy successfully suppressed xenograft tumors in 18 days without distal harm when subjected to a single 15 min near-infrared (NIR) laser treatment. This sophisticated GQD nanotheranostic with the capability of image tracking, enhanced tumor accumulation, NIR-triggered tumor penetration and hyperthermia ablation for photothermal-chemo therapy boosts tumor treatment and could be even potentially used in other biological applications.

Session No.: P3-080

Wrinkle Pattern-based Reverse Transfection System for Efficient Sirna Delivery

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Small interfering RNA (siRNA) has been emerging therapeutic source through silencing target genes involved in disease progress. In addition, siRNA is useful tool in fundamental biology to reveal cellular mechanism or function of proteins. Thus, the researches developing siRNA delivery systems have been widely conducted, however most of these researches have focused on design of delivery materials such as cationic polymers, liposomes, or inorganic nanoparticles and often overlooked the importance of delivery method. Therefore, herein, we developed a reverse transfection system for efficient siRNA delivery using wrinkled surface functionalized with siRNA-lipidoid nanoparticles. In contrast to conventional siRNA delivery method, forward transfection (solution-mediated transfection) system, reverse transfection (surface-mediated transfection) method delivers siRNA to cells by seeding target cells onto siRNA-loaded surface. This reverse transfection method could provide cells with a higher chance to contact siRNA and allow for extended siRNA exposure to cells, leading higher transfection efficiency. We introduced wrinkle pattern on the reverse transfection system to further enhance transfection efficiency by increasing contact area between surface and cells. siRNA-lipidoid nanoparticles were immobilized on wrinkled surface via catechol-mediated coating. Compared to flat substrate, wrinkled substrate exhibited increased reverse transfection efficiency. Furthermore, due to a high flexibility of wrinkle fabrication procedure, we applied wrinkles into microwell structures which enables high-throughput screens of siRNA transfection. Therefore, our wrinkled microwell systems could provide highly efficient high-throughput siRNA delivery platform.

Session No.: P3-081

Developing Biodegradable Particles as a Delivery Platform for Therapeutic Agents

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In regenerative medicine, biodegradable particles could be used as a delivery platform for bioactive substances. The release of therapeutics agents from particles can target and control the behavior and the fate of the cells. Among the drug carriers, microparticles formulated from polyketals have been used as delivery vehicles for degenerative diseases because they have excellent biocompatibility and do not generate inflammatory acid degradation products as do polyester-based biomaterials. In this study, polyketal microparticles (PKMs) which contain hydrophobic compounds (chrysin and magnolol) were prepared via single emulsion method. Various formulations and sizes of PKMs were prepared and characterized to optimize their release rate. More importantly, delivery of these drug loaded microparticles significantly reduced the NO production and TNF-alpha production in lipopolysaccharides (LPS)-activated macrophages. We expected drug loaded PKMs will enhance the bioavailability of magnolol and chrysin, and will have numerous clinical applications.

Session No.: P3-082

Development and Application of Micro Polysaccharide Drug Carriers with Incorporation of Doxorubicin and Spio for Targeting Treatment of Hepatocellular Carcinoma

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In this study, we developed a novel polyelectrolyte micorparticle, DOX-SPIO-CS/CHI MP, as a drug delivery system (DDS) for hepatic cancer treatment. Also, we investigated the properties of these microparticles, such as material characterizations, formulation tests, in vitro study, and in vivo study. The results show that our DOX-SPIO-CS/CHI MPs displays an average diameter of 110.7 ± 30.23 nm, and reveals a spherical shape. The encapsulation efficiency of drug carrier is approximately 31% (SD = 8.07) based on our spectroscopic measurement. In the results of release profile test, we found a sustained-release behavior of DOX-SPIO-CS/CHI MPs, which released 51.5% of DOX within 48 h of testing time. Based on the results of cell viability assay and animal study, the DOX-SPIO-CS/CHI MP was found to show stronger cytotoxicity than that of free DOX, when it was given to Hep G2 and Huh-6 human liver cancer cell line in vitro and nude mice of Hep G2/Huh-6-bearing mice model in vivo.

Session No.: P3-083

Dextran-based Nanogels with pH/Redox Dual Responsiveness as Efficient Anti-cancer Drug Carriers

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Anti-cancer drug delivery systems (ACDDS) with dual responsivities to the tumor microenvironment attracted increasing interests owing to their excellent performance in reducing side-effects of drug. Therefore, more and more effords were put into developing effecient fabrication methodology of ACDDS. In the current study, polyacrylic acid (PAA) grafted dextran (Dex) nanogels (NGs) with covalent crosslinked structure bearing redox sensitive disulfide crosslinking points (SS), denoted as Dex-SS-PAA NGs, were synthesized efficiently through a one-step self-assembly assisted methodology. The Dex-SS-PAA NGs were subsequently conjugated with doxorubicin (DOX) through an acid-labile hydrazone bond (Dex-SS-PAA-DOX). The anti-cancer activity of Dex-SS-PAA-DOX was tested in both tumor cells in vitro and tumorgenesis model in vivo. The results revealed that the Dex-SS-PAA-DOX NGs exhibit a controlled release of DOX which could be triggered by acidic and reducing environments. Moreover, MTT cell viability assays revealed that the Dex-SS-PAA-DOX NGs exhibited a strong ability to inhibit the growth of human breast cancer MDA-MB-231 cells, but low toxicity toward normal cells. The anti-cancer activity of the DOX-loaded NGs as tumor inhibiting agents toward nude mice bearing subcutaneous MDA-MB-231 tumor xenografts demonstrated that the Dex-SS-PAA-DOX NGs greatly reduced the toxicity of free DOX, while retaining its high anti-cancer effects. Our study demonstrated that the Dex-SS-PAA-DOX NGs were very promising as a drug delivery system for anti-cancer therapeutics, and the one-step self-assembly assisted methodology were flexible and highly efficient in fabricating dextran-based ACDDS.

Session No.: P3-084

The Effects of EGCG on ROS and NO Release Under Hypertension: One of the Possible Cues Focused on Mitochondria

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Hypertension is a condition associated with low nitric oxide (NO) releasing, high reactive oxidative stress (ROS) level and activated inflammation. EGCG (Epigallocatechin gallate), an anti-oxidant in green tea, has shown protective effects on hypertension. However, most studies were based on animal model or clinical data, remaining a relative blank area in vitro study and unclear mechanisms. To mimic hypertension status, endothelial cells (ECs) were subjected to hydrostatic pressure with differential magnitude and duration. NO assay and inflammatory factors were conducted to confirm hypertension condition. Our results showed 200 mmHg static hydrostatic pressure decreased NO releasing, and activated inflammatory markers significantly, which indicated analogous hypertension status. Then we measured ROS levels in mitochondria (mROS) and NO release from two groups depending on pre-treatment by EGCG or not. Without EGCG pre-treatment, release of NO was decreased, while mROS was found to be increased under hypertension condition. With EGCG pre-treatment, we found level of NO release was increased, comparable to that in control group. Also mROS was found to be decreased in treated group. In conclusion, the present study developed a hypertension model in vitro and showed the protective potential of EGCG for hypertension treatment through reducing oxidative stress from mitochondria. Especially the ROS level in mitochondria in relation to hypertension and related status was evaluated for the first time. This study under analogous hypertension status in-vitro can contribute to the study on effect of EGCG on early protection from what hypertension may causes. This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea(NRF-2014H1C1A1073148) and by Priority Research Centers Program (2010-0020224, the Ministry of Education, ROK).

Session No.: P3-085

Testing Method Development and Validation for in Vitro Skin Irritation Testing (SIT) by Using Reconstructed Human Epidermis (RHE) Skin Equivalent - EPiTRI

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Topical exposure to chemicals and cosmetic products can lead to various adverse skin effects. Corrosion and irritation are commonly regarded as two major categories among these adverse effects. Corrosive substances irreversibly damage the skin beyond repair, while irritant substances lead to a reversible local inflammatory reaction caused by the innate (non-specific) immune system of the affected tissue. Current regulatory requirements focus on the assessment of the acute irritation potential of chemicals and cosmetics in order to support the risk management. Internationally accepted test methods for skin irritation testing (SIT) include the traditional in vivo animal test as well as in vitro test methods. However, there is a trend changed from in vivo to in vitro testing due to the 3R (replacement, reduction, refinement) requirement resulted from recent animal testing ban. All accepted in vitro test methods are based on the RhE technology (Reconstructed human Epidermis) validated by ECVAM. RhE models use normal human keratinocytes to form a multi-layered epidermis including a stratum corneum at the top and can function as a barrier. There are only few RhEs which have been validated by ECVAM for SIT and none of these are of Chinese heredity. ITRI started the RhE project some years ago based on our culture experience of cells isolated from human donors, the cell expansion technology, and our own GTP/GMP facilities. So far, a multi-layered epidermis with reproducible barrier function was developed and used for SIT in accordance to OECD439 guideline. In this presentation, we reported the progress for developing the SIT method. An overall sensitivity of 100%, specificity of 70% and accuracy of 85% was obtained in the Phase I validation. The study shows that EPiTRI could provide as an in vitro model to evaluate the skin irritation and a reliable SIT method has been developed accordingly.

Session No.: P3-086

Differentiation Control of Adipogenic Progenitor Cell Line, 3T3-L1, Through the Regulation of Protein Adsorption on Poly(2-Methoxyethyl Acrylate) (PMEA) Analogs.

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Stem cells have recently attracted great interests for regenerative medicine. There are many approaches to control stem cell differentiation by the development of new cell culture substrates. For this purpose, synthetic polymers have been focused although synthetic polymers exhibit weak ability to control stem cell differentiation due to the lack of biological signal molecules. Generally, the cells adhere on synthetic polymer substrates through the interaction with adsorbed proteins on the substrates. This interaction activates intracellular signals to control various cellular functions including stem cell differentiation. Therefore, it is expected that stem cell differentiation might be controlled by the regulation of protein adsorption on synthetic polymer substrates. We have reported that protein adsorption behavior is altered on the substrates coated with poly(2-methoxyethyl acrylate) (PMEA) and its analogs. Also, we have reported that PMEA analogous substrates can support cell adhesion. Here, we examined adipogenic differentiation of 3T3-L1, an adipogenic progenitor cell line, on PMEA analogous substrates. Four types of PMEA analogs (PMEA, PMe2A, PMe3A, and PTHFA) and poly (2-methacryloyloxyethyl phosphorylcoline-co-n-butyl methacrylate) (PMPC) were spin-coated with polyethylene terephthalate (PET) discs and were used as cell culture substrates. The amounts of adsorbed proteins were in the order of PTHFA > PMEA, PMe2A, PMe3A >> PMPC. 3T3-L1 cells adhered on all examined PMEA analogous substrates and PET substrate but not on PMPC substrates. 3T3-L1 adhered on PMEA, PMe2A, and PMe3A through both integrin-dependent and –independent mechanisms although the cells adhered on PET and PTHFA through only integrin-dependent mechanism. Additionally, 3T3-L1 exhibited the greater adipogenic gene expression levels of Fasn and Gpd2 on PMEA, PMe2A, and PMe3A substrates than did that on PET and PTHFA substrates. Conclusively, PMEA analogous polymers are expected to be preferred cell culture substrates for adipogenic differentiation control of adipogenic progenitor cells and mesenchymal stem cells.

Session No.: P3-087

Stem Cell Culture Using Honeycomb-patterned Polymer Film

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Induced pluripotent stem cells (iPS cells), which can differentiate into derivatives of all 3 germ layers (endoderm, mesoderm, and ectoderm), are a promising cell source for various applications, such as, regenerative medicine, pharmacological studies, and fundamental studies of cell differentiation. The cell-material interactions are one of important factors to regulate the differentiation fates of iPS cells. In this study, we investigated the effect of material topography on the properties of mouse iPS cells. To evaluate the topographic effect of material, we developed a honeycomb- patterned polymer film (HF substrate), which had a regular honeycomb-patterned porous structure at micro-scale in the film, as a new scaffold. The properties of mouse iPS cells on the HF substrate were compared with a flat surface film without porous structure (FF substrate). Although the cells adhered to the surfaces of both substrates, the adhesion of cell-substrate was weak and many cells formed embryoid bodies that were three-dimensional aggregates, with the increase of culture period. The cell proliferation on HF substrate was lower than that on FF substrate, indicating that the HF substrate represses the cell proliferation. The expressions of early endoderm markers in both substrates were almost the same. However, the expression of early mesoderm markers in HF substrate was higher than that in FF substrate. This result suggests that the HF substrate promotes to differentiate into mesoderm lineages. From these results, we demonstrated that the properties of stem cells were influenced by the topography of substrate.

Session No.: P3-088

Effect of Oxygen Environment on Embryoid Body Culture of Mouse iPS Cells

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Induced pluripotent stem cells cells exhibit an infinite self-renewal capacity and pluripotency, and therefore, they are a promising cell source for regeneration medicine and drug discovery research. The formation of embryoid bodies (EBs) is one of culture methods to promote the initial differentiation of iPS cells. As a culture platform for generating EBs, we have developed a microwell chip which the microwells of several hundred micrometers were regularly fabricated on a culture substratum. It allowed the production of a large number of homogenous EBs of desired size. In this study, we prepared two similar microwell chips which made from poly-methylmethacrylate (PMMA; oxygen non-permeable material) and poly-dimethylsiloxane (PDMS; oxygen permeable material), and evaluated the effects of oxygen environment on the EB properties of mouse iPS cells. The iPS cells formed EBs in each microwell in both chips within 1 d of culture, and the EBs grew via cell proliferation with the increase of culture period. The growth of EB size in the PDMS chip was higher than that in the PMMA chip. The differentiation patterns of EBs were compared at 7 d of culture. The expressions of hepatic (TTR and AFP) and cardiac (Nkx2.5 and α-MHC) markers in the PMMA chip were higher than those in the PDMS chip. In contrast, the expression of vascular markers (Flk1 and PDGFRβ) in the PMMA chip was lower than that in the PDMS chip. These results indicated that the oxygen environment is important factor to control cell proliferation and differentiation fate of mouse iPS-EB.

Session No.: P3-089

Differentiation Properties of Mouse iPS Cells on Poly-dimethylsiloxane Substratum

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Stem cells including embryonic stem cells (ES cells), induced pluripotent stem cells (iPS cells), and mesenchymal stem cells (MSCs) are a promising cell source for regeneration medicine and drug discovery research. The differentiation fates of such stem cells are regulated by various factors, such as intercellular signaling, cell-cell contact, mechanical force, and cell-material interaction. In this study, we focused on the cell-material interactions. Poly-dimethylsiloxane (PDMS), which is elastomer and has high gas permeability and biocompatibility, is a unique material for various applications including cell cultures and micro-devices, and we investigated the differentiation properties of mouse iPS cells on a PDMS substratum in this study. Mouse iPS cells were cultured on the PDMS substratum with flat surface, which was prepared by curing reaction of pre-polymer. A polystyrene substratum with tissue culture surface (TC substratum) was used as a control condition. The iPS cells adhered to the surfaces of both substrata and proliferated with the increase of culture period. Although the cells in the TC substratum showed the spreading morphology for the culture duration, three-dimensional colony morphology was formed in the PDMS substratum. The differentiation patterns of iPS cells were compared at 7 d of culture. There were no significant differences in the expressions of mesoderm gene markers. However, the expression of hepatic gene markers in the PDMS substratum was higher than that in the TC substratum. This result suggests that the properties of iPS cells are influenced by the difference of culture material and that the PDMS substratum promotes to differentiate into endoderm lineages.

Session No.: P3-090

Evaluation of Bone Regeneration Through Plasmid DNA-releasing Hydrophilized PCL Membrane with Stem Cells Using Mini-Pig Model

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Bone morphogenetic protein-2 (BMP-2) has been proven to be an effective stimulant for the osteogenesis of stem cells in bone tissue engineering. However, its short half-life and the inability to restrict the protein to a localized area are still remained as limitations. Recently, the bone regeneration using tissue engineering technique and local gene delivery system have been considered as one of the promising therapeutic methods. We prepared plasmid DNA (with BMP-2 encoding) complex-loaded polycaprolactone (PCL)/Pluronic F127 membrane to enhance the osteogenic differentiation of adipose stem cells (ASCs). The pDNA complex was efficiently loaded into the membrane (> 80% of initial loading amount) and continuously released from the membrane for a long period of time (more than 3 months). From the in vitro transfection assay, it was observed that the pDNA complex released from the membrane was efficiently transfected into the ASCs, inducing the ASCs to secrete BMP-2, increase calcium deposition and gene expressions of alkaline phosphatase, runt-related transcription factor 2 and type I collagen, and finally inducing the ASC differentiation into osteoblasts effectively. From the in vivo animal study using a mini-pig model, pDNA complex-loaded PCL/F127 membranes showed faster bone regeneration than the control (blank) and PCL/Pluronic F127 membrane without plasmid DNA. From the results, we could suggest that the pDNA complex-loaded PCL/F127 membrane can be a good candidate as a gene delivery system for osteogenesis of ASCs and bone regeneration, owing to its high gene transfection efficiency and low cytoxicity as well as long-term delivery of BMP-2.

Session No.: P3-091

Plasmid DNA-loaded Injectable Bulking Agent System for Vocal Fold Augmentation

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General vocal fold paralysis causes incomplete vocal fold closure and it results in dysphonia and dyspnea. Various techniques have been proposed for the treatment of vocal fold paralysis. Recently, the injection laryngoplasty techniques for managing of glottal insufficiency have gained popularity because of its initial low cost, minimal invasiveness, and low morbidity as compared to surgical repair method. A number of injectable substances have been adapted in the clinical practice, how-ever, relatively short-term volume stability (by migration and resorption), inflammation, granuloma formation, and decreased vocal fold vibration are considered as limitations for practical use. In this study, we prepared plasmid DNA (encoding for bFGF) complex–loaded alginate/hyaluronic acid (HA) hydrogel dispersed with polycaprolactone (PCL) microspheres, as an injectable bulking agent for the treatment of vocal fold paralysis. For effective transfection of plasmid DNA into stem cells, the plasmid DNA was condensed by polyethyleneimine-polyethylene glycol (PEI-PEG). The pDNA complex was effectively loaded into the alginate/HA hydrogel and was continuously released from the hydrogel for 35 days. We investigated the transfection efficiency of pDNA complex into adi-pose-derived stem cells (ADSCs) and its cytotoxicity, bFGF expression from the gene-transfected cells, and the proliferation/differentiation behavior. From the in vivo animal study, the bulking agent showed significantly fast tissue regeneration. From the results, the pDNA-loaded injectable bulking agent may be a good system for the treatment of vocal fold paralysis.

Session No.: P3-092

Effect of Conditioned Media and TGFβ3 Treatment on Adult Stem Cells for Meniscus Tissue Engineering

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For stem cell-based meniscus tissue engineering, we employed tonsil-derived mesenchymal stem cells (T-MSCs), a type of adult stem cells obtained from tonsillectomy, and COL-RF-HA hydrogel, a biomimetic scaffold conducive to meniscus ECM formation. Prior to encapsulation, T-MSCs were primed for two passages with conditioned media (CM) from meniscal fibrochondrocytes. T-MSCs (P6) were encapsulated in COL-RF-HA hydrogel and cultured in vitro for three weeks in chondrogenic media, with or without TGF-β3. Based on different media treatment before and after encapsulation, cell-laden constructs were divided into four groups: GM-TGFβ3, GM+TGFβ3, CM-TGFβ3 and CM+TGFβ3. Real-time PCR, biochemical and histological analyses suggest that CM+TGFβ3 had significantly higher fibrocartilage-related gene expression level, cell proliferation and GAG accumulation compared to the other groups. By comparing results of GM+TGFβ3 and CM-TGFβ3, we were able to assess the effects of CM and TGF-β3 independently; conditioned media more effectively induced T-MSCs to form meniscus ECM than growth factor. In vivo experimentation was carried out via two animal models: mouse subcutaneous implantation and rabbit meniscal defect. Cell-laden constructs were pre-cultured with or without TGF-β3 for a week, implanted in nude BALB/c mice and New Zealand white rabbits, and kept in the respective animal models for 6 and 10 weeks. In vivo results were consistent with our hypothesis as well as in vitro results. Thus, CM+TGFβ3 has the greatest potential for stem cell-based meniscus tissue engineering.

Session No.: P3-093

Transgelin Contributes to Migration and Invasion in Hepatocellular Carcinoma Cells

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Cancer stem cells (CSCs) have established notorious evaluation for their ability to self-renew, hence this leads in the initiation of malignancy, promotion of tumor formation and metastasis. In this study, we sorted subpopulations of cells from the hepatocellular carcinoma cell-line, Huh-7 using CD133 marker. The CD133+ cells have exhibited self-renewal and stem cell characteristics by colony forming assay and sphere culture. In our two-dimensional gel electrophoresis (2-DE) and ESI-Q-TOF MS/MS experiment which both CD133+ and CD133- were used, significant different expression between the samples were detected. Transgelin which has been implicated in cell migration was highly increased in CD133+ cells than CD133- cells. We also detected that the highly expressed levels of Transgelin considerably increased the invasive potential of tumorigenic cells, while lowly expressed levels decreased the invasiveness. Moreover, we observed that Transgelin was co-expressed with CXCR4, dependent tumor growth and invasion of cancer cells. Our study could provide clues for metastatic potential of CSCs arises from highly expressed Transgelin. It is important to elucidate the cellular mechanism affecting their different and specific functions.

Session No.: P3-094

Nanotopographic Influence on the in Vitro Behavior of Induced Pluripotent Stem Cells

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The purpose of this study was to identify nanopatterns that can influence the stemness and proliferation as well as the adhesive properties in iPSCs and explore the feasibility of applying these nano-features for regenerative medicine. Three kinds of nanopattern were fabricated from PDMS membranes, irregular patterned membrane (IPM), groove patterned membrane (GPM) and post patterned membrane(PPM) in addition to flat patterned membrane (FPM) which did not have any nanotopographic features. During in vitro culture, iPSCs showed tendency for aggregation, and did not spread out well on the surfaces of GPM or PPM at passage 1. However, with continued passaging, the tendency to form aggregates was greatly reduced. Calculation of population doubling time showed that while GPM and PPM had low values compared with FPM and IPM at P1, the differences disappeared in later passages. The expression of Ki67 in iPSCs gradually decreased with continued passaging in cells cultured on FPM and IPM, but not in those cultured on GPM and PPM, being significantly higher on GPM and PPM than FPM or IPM at P6 and P10. The expression of Oct3/4 and Nanog was significantly higher on GPM and PPM than on FPM at P6 and P10. At P5, numerous filopodia were demonstrated in the peripheral attachments of iPSC colonies on FPM and IPM while GPM and PPM generally had globular appearance. The expression of the focal adhesion molecules was significantly greater on GPM and PPM than on FPM and IPM at P6 or P10. In conclusion, continued passaging on regular nanopatterns including groove- and post-forms were very effective in maintaining an undifferentiated state and proliferation of iPSCs. ACKNOWLEDGEMENTS: This study was supported by the National Research Foundation (NRF) funded by the Korean government (2015R1A2A1A09002793 and 2013R1A1A2062961).

Session No.: P3-095

Selection of Human Glioblastoma Cancer Stem-like Cells by Using Mixed Hydrogel Culture System

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Cancer stem cells (CSCs) has a number of functions, it represents a subpopulation of tumor cells. These cells processed the ability of self-renewal, tumor initiation, disease relapse or metastasis, and resistance to radiotherapy and chemotherapy. However, The biggest problem of CSCs research is how to isolate CSCs from solid cancer and maintain their survival. Herein, hydrogel materials composed of agarose and hydroxypropyl methyl cellulose (HMC) in different concentrations were established and simulated with brain carcinoma CSCs microenvironments for CSCs selection with a label-free culture system. Hydrogel materials in different concentrations could provide culture condition variation, like stiffness variation, nutrient gradient variety, and oxygen concentration difference for glioblastoma cells. When cultured on hydrogel materials, human glioblastoma cell lines aggregated and formed colony automatically after 4 days of cultures. The expression of CSCs markers, such as CD133 of these cancer stem-like cells isolated from hydrogel-based culture system will be examined by using flow cytometry. Cell apoptosis were evaluated by flow cytometry Annexin V and PI double staning, it can detect the externalization of phosphatidylserine in apoptotic cells. Results of cell apoptosis analysis is consistent with the results of drug resistance test. Finally, the stem cell related genes expression of selected cells from agarose-HMC culture system after 4 and 6 days culture will be testing by RT-PCR, these gene include stem cell gene Nanog, Oct-4, Sox-2 and drug resistance gene ABCG2. Therefore, according to the results, it is supposed that microenvironments selection and colony formation on a mixed hydrogel system may provide a label-free CSCs selection strategy and drug testing model for future biomedical applications.

Session No.: P3-096

Microencapsulation-induced SOX9-DNA Binding Interaction in Mediating Mesenchymal Condensation and Chondrogenesis

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Chondrogenesis of human mesenchymal stem cells (MSCs) apparently include a crucial transitional stage called mesenchymal condensation (MC), which is complex and governed by specific transcription factors (TFs). Among these, SRY-Box 9 (SOX9) plays pivotal roles in mediating cell-cell and cell-matrix interactions during MC and chondrogenesis. In this study, we aimed at investigating the association of SOX9-DNA binding activity with specific chondrogenic markers, collagen type II (COL2A1) and aggrecan (ACAN), in 3D-microencapsulated hMSCs and demonstrated the time course for expression pattern and level for chondrogenic markers at 2.5e5, 5e5 and 1e6 cell densities. Under microencapsulation, it was visibly observed nuclear localization of SOX9 at 1-h, 1-d and 2-d post-microencapsulation and higher COL2A1 deposition with time and cell density. For chondrogenesis, results from qPCR data normalized by monolayer coated with collagen (2Dc) revealed the highest SOX9 expression at 1-h post-microencapsulation and a gradual reduction with time. Concordantly, ACAN showed a decreasing trend with time in spite of lower expression than 2Dc in the entire duration. Unlike ACAN, COL2A1 expression increased at early time-points, then fluctuated with time and reached a maximum at 7-d. Two-way ANOVA showed significant differences for SOX9, ACAN and COL2A1 separately in the three cell densities ($p<0.0001$ for SOX9 and ACAN; $p=0.003$ for COL2A1) and at the seven time points except COL2A1 ($p<0.0001$). Furthermore, chromatin-immunoprecipitation (ChIP) analyses exhibited stronger SOX9 binding activity with the promoter and intron-1 enhancer of COL2A1 in MSCs with 3D-microencapsulation than 2Dc. Altogether, this work suggests MC and chondrogenesis are presumably promoted by enhanced SOX9 binding activity due to microencapsulation-induced epigenetic modification on the regulatory sites of chondrogenic markers. Further studies on the interaction between 3D microencapsulation and SOX9 binding activity are warranted to a more understanding of the transcriptional regulation for hMSCs chondrogenesis.

Session No.: P3-097

Exosomes Released from Wharton's Jelly Mesenchymal Stem Cells Promote Angiogenesis and Vascular-Like Structures Stabilization of Endothelial Cells

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Mesenchymal stem cells (MSCs) has been indicated as a powerful therapeutic resource for a diversity of degenerative diseases. Most of these pathologies are linked to insufficient blood flow supply. Besides renovate of tissue function by the differentiation potential of MSCs, the paracrine effects of MSCs participate in neovascularization are also demonstrated in many studies. Exosomes are a group of microvesicles released from cells via exocytosis pathway. These small extracellular vesicles can function as carriers of various proteins and genetic materials. Therefore, exosomes have been recognized as a potent mediator involved in many biological functions. However, the role of exosomes in the management of angiogenesis remains to be established. In this study, we isolated exosomes from Wharton's Jelly MSCs (WJ-MSCs) and analyzed their characteristics and angiogenesis capability. WJ-MSCs derived exosomes can facilitate the migration of endothelial cells and stabilizing the vascular-like structures of human umbilical vein endothelial cells (HUVEC) in vitro, and the precise molecular mechanisms that trigger angiogenesis by WJ-MSCs derived exosomes will be further investigated. Through this study, we may provide a new insight into angiogenic therapies with stem cell-derived exosomes.

Session No.: P3-098

Migration of Lung Cancer Cells in a 3D (Bio-mimetic) Environment with Simulated Microgravity

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A metastasis of cancer cells is one of the most important factors at the cancer therapy. Therefore, many people have studied the cancer migration to understand cancer biology. Recently, it has been reported that a migration speed is modulated by a variation of an external environment. Especially, a behavior of cancer cells in microgravity condition has gained attention as a tool for development of a new cancer therapy. In this study, we estimated the migration of two types of lung cancer cell lines (A549, H1703) using bioreactor with microgravity. When we measured the cell proliferation (24hrs and 48hrs) with / without microgravity, a proliferation rate of A549 with microgravity was faster than that of control group. At the scratch test of A549 based on the wound healing model, wound widths in microgravity were reduced quickly. Although H1703 in microgravity environment showed an enhanced decrease of wound width than that of control group, migration speed of H1703 was slower than that of A549. Gene expression levels of each condition at the 24hrs and 48hrs showed the similar trends with wound healing results. From the results, we could supply a new platform for tissue culture as well as cancer study.

Session No.: P3-099

Non-Viral Direct Lineage Reprogramming of Fibroblasts into Highly Functional Neuron-like Cells

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Recent studies have shown that defined combinations of factors can directly reprogram terminally differentiated cells into another lineages without passing through a pluripotent stem cell-like state. Wernig et al. first described the direct conversion of fibroblasts into functional induced neuronal cells using three developmental transcriptions i.e. Brn2, Ascl1, Myt1l (BAM factors). Subsequent successful reports of direct lineage reprogramming into various neuronal subtypes have rapidly expanded this field and have endorsed the prospect of autologous cell replacement therapy for neurodegenerative disorders. So far, all the studies had used viral vectors to deliver the transcriptional factors, which has an advantage of high transfection efficacy but is imposed to the safety concerns, therefore limits the scope of their potential clinical applications. Here, we report a novel method for reprogramming primary mouse embryonic fibroblasts directly into multipotent and neural lineage restricted induced neuronal-like cells by non-virally delivering plasmids encoding BAM factors with biodegradable polymer nanoparticles. These induced neuronal cells uniformly displayed morphological and molecular phenotypes of neuronal cells with mature neuron-like electrophysiological functions. Therefore, our approach employing nonviral delivery method can provide efficient, safe methodology for improving direct reprogramming to generate neuronal lineage cells for therapeutic applications. This study was supported by grant (2015R1A2A1A15053771) from the National Research Foundation (NRF) of Korea.

Session No.: P3-100

Influence of Nano-grooved Topography and Chemical Surface Properties on Differentiation of Neural Cells

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The differentiation of stem cells into neural cells are governed by biomolecular and matrix topographic cues. and bioactive molecules can generate physico-chemical cues that control proliferation and differentiation of neural cells. In this study, we investigated the neural differentiation of Pheochromocytoma 12 (PC12) cells, adipose stem cells and stem cells from umbilical cord blood on nano-grooved surfaces of which dimensions of 400 and 800 nm in width and 100 and 400 nm in depth. The substrates were first coated by polydopamine in order to increase cell affinity. The cells were cultured on the nano-grooved surfaces in the presence of neural growth factor. We found that the cells aligned well with the aspect of the grooves, in contract to random orientation of the cells on the flat controlled. Furthermore, the lengths of neuritis were longer on the cells cultured on the grooved surfaces. Our results indicated that the depth of grooves is more important to neural differentiation than the width. In conclusion, nano-grooved topography, along with neural induction agents supported PC12 cells neurites outgrown and differentiated into neuron-like cells.

Session No.: P3-101

Generation of Cardiac Lineage-primed and Paracrine Profile-improved MSCs by Magnetic Nanoparticle-driven Co-culture for Myocardial Infarction Treatment

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Electrophysiological phenotype development and paracrine secretion of mesenchymal stem cells (MSCs) are the major factors of their therapeutic efficacy for heart diseases, such as myocardial infarction (MI). In such respect, co-culture of MSCs with cardiac cells has windowed a platform for cardiac feature development of MSCs. From the previous studies, active gap junctional crosstalk of MSCs with cardiac cells in co-culture has been known to play a major role in the MSC modification. Here, we report that iron oxide nanoparticles (IONPs) can deliver Fe2+ metal ions into cardiomyoblasts (H9C2) for intercellular signaling modification and augment the expression of gap junction protein connexin 43 (Cx43), which would be critical for gap junctional communication with MSCs in co-culture for the generation of therapeutic potential-improved MSCs. MSCs co-cultured with IONP-laden H9C2 (co-cultured MSCs: cMSCs) showed active cellular cross talk with H9C2, and displayed significantly higher levels of cardiac biomarkers and a cardiac repair-favorable paracrine profile, both of which are crucial for successful MI repair. Intramyocaridal injection of cMSCs significantly improved animal survival and heart function compared with the injection of unmodified MSCs. The present study highlights an application of IONPs in developing gap junctional crosstalk among the cells, and generating cMSCs that exceeds the reparative potentials of conventional MSCs.

Session No.: P3-102

Hypoxia-conditioned Adipose Mesenchymal Stem Cell Medium Impacts on Salivary Glandular Cell Survival and Function After Irradiation in Organotypic Three-dimensional Culture

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INTRODUCTION: Mesenchymal stem cells (MSC) have been reported to ameliorate irradiation-induced salivary gland hypofunction through trophic effects and immunomodulation. We investigated the radioprotective effects of MSC secretome could be enhanced by hypoxia preconditioning in an organotypic three-dimensional (3D) co-culture model. METHODS: Human parotid gland epithelial cells (hPEC) were cultured on Matrigel to form 3D acinar-like spheroids, which were followed by irradiation. Human adipose mesenchymal stem cells (hAdMSC) were cultured under normoxia (NMX, 20% O2) or hypoxia (HPX, 1% O2) condition for 24 hours and then co-cultured with the irradiated 3D spheroids. RESULTS: IR induced the destruction of structural integrity and decreased salivary secretory function of the 3D spheroids. The irradiation-induced structural damages and phenotypic changes were ameliorated by coculture with HPX-conditioned hAdMSC. The coculture with HPX-conditioned hAdMSC significantly attenuated the loss of epithelial polarity and acinar-specific cellular functions of the 3D spheroids to secrete α-amylase in response to Ca2+ agonists. IR-induced apoptosis of the 3D spheroids was also reduced by HPX-conditioned hAdMSC, which was associated with an decreased expression of PTEN and p53, along with an increase in AKT, MDM2 expression. Protein assay revealed that HPX-conditioned hAdMSC secreted more FGF7 and FGF10 than NMX-cultured hAdMSC. IR-induced apoptosis was significantly attenuated by FGF7 and FGF10. DISCUSSION & CONCLUSIONS: Our results suggest that HPX-preconditioning promotes hAdMSC to produce more FGF7 and FGF10, which protect salivary hypofunciton by attenuation of IR-induced apoptosis via the mechanism of modulation of PTEN/AKT/p53.

Session No.: P3-103

Evaluation the Anti-inflammation and Anti-apoptosis Abilities of Mesenchymal Stem Cell by Chondrocyte/ Wharton's Jelly Mesenchymal Cells (WJMC) Co-Culture System

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Due to mesenchymal stem cell (MSC) was easily expanded in culture, generally not tumorigenic, and can be readily obtained from patients, they had become an ideal cell source for osteoarthritis (OA) therapy. Here, we tried to use human Wharton's Jelly MSC (WJMSC) as a cell source since it could be collected from umbilical cord and cryopreservation for allogeneic or autologous use. Chondrocyte was induced inflammation by hydrogen peroxide (H2O2) incubation, and then co-cultured with WJMSC or conditioned medium to evaluate the anti-inflammatory and anti-apoptosis ability. Four kinds of culture conditions would be evaluated in the study including (I) H2O2-chondrocyte/ WJMSC co-culture system, (II) WJMSC conditioned medium system, (III) H2O2-chondrocyte and (IV) chondrocyte control group. In system I, chondrocyte and WJMSC was cultured on the bottom layer and the transwell, individually. In system I, we found when chondrocyte/WJMSCs ratio was decreasing, the inflammation genes such as COX-2, MMP13 and iNOS were also significantly up-regulated. Besides, the results of 10X conditioned medium treated group (system II) were also similar to the system I, only IGF-1 was significant higher than others. However, the inflammation genes and apoptosis genes were significantly down-regulated by 10X pass through medium addition after 1-day incubation. Summarized the cell viability, cell morphology and gene expression results, chondrocyte treated with 200 μM H2O2 is the optimal concentration to induce the cell damage. We also found 10X WJMSC pass through medium possess anti-inflammation ability and assist chondrocyte repair from the damage of H2O2. It is shown that 10X WJMSC pass through medium not only reduce the inflammation (COX-2, MMP13, iNOS, and TNF-alpha but also decrease the apoptosis related gene expression such as Caspase 3, TNF-R1 and Caspase 8. Compared to all of the co-culture systems, the 10X WJMSC pass through medium could effective reduce inflammation and apoptosis.

Session No.: P3-104

Improved Skin Flap Survival in Venous Ischemiareperfusion Injury with the Use of Adipose-derived Stem Cells

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Introduction: The purpose of this study was to investigate the efficacy of stem cell therapy as an adjuvant treatment for congested skin flap. Method: Sprague-Dawley rats (n521) were randomized into three groups. In group I, the flap was sutured without venous ischemia. In group II, the vein was selectively clamped for 4 hours, and complete medium was administered upon clamp removal. In group III, ADSCs were administered upon removing the clamp. On postoperative day 7, the survival areas and the histopathologic findings were assessed. In addition, the expression of heme oxygenase (HO)-1 and nuclear factor (NF)-kB was assessed using immunofluorescent staining and western blot analyses. Results: Compared with group II, group III showed significantly increased flap survival (31.2%611.9% vs. 51.6%613.6%, P<0.05). The degree of histological abnormalities was significantly lower in group III than in group II (9.38%61.39 vs. 6.46%62.57, P<0.05). In addition, in group III, the expression of NF-jB was significantly lower (0.5160.21 vs. 0.3460.21, P<0.05), whereas that of HO-1 was significantly higher (0.2560.11 vs. 0.4360.18, P<0.01). Immunofluorescent staining also showed more HO-1-positive cells in group III than in group II (10.9%61.6% vs. 16.0%61.7%, P<0.01). Conclusion: Our study demonstrated that treatment with ADSCs significantly increased flap survival in venous ischemia-reperfusion conditions. Further investigation of these protective effects and optimization of the treatment protocol could make cell therapy a viable treatment.

Session No.: P3-105

Effects of Bone Grafts on the Proliferation and Osteogenic Differentiation Human Gingival Derived Mesenchymal Stem Cells

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The dimension of alveolar ridge is decreased by bone atrophy and pneumatization of the maxillary sinus after loss of teeth in the posterior maxilla. Various osteo- conductive materials have been used to augment the sinus floor, but materials are cells free. Attempts use mesenchymal stem cells combination with an osteoconductive scaffold. Adult stem cells that can be derived from various tissues, including bone marrow, umbilical cord, fat, dental pulp and gingival tissue. This study was to isolate human mesenchymal stem cells (MSCs) from the gingiva (GMSCs) and confirm their multiple differentiation potentials. GMSCs culture were analyzed for cell shape, cell cycle, colony-forming unit-fibroblast (CFU-F) and stem cell markers. Cells were then induced for osteogenic, chondrogenic and adipogenic differentiation and analyzed for differentiation markers (mineralization nodule formation and Runx2, ALP, osteocalcin (OCN) and collagen I expressions for the osteogenic differentiation, and lipid vacuole formation and PPAR γ -2 expression for the adipogenic differentiation, and proteoglycans (PGs) formation and collagen II, aggrecan expression for the chondrogenic differentiation). This study aimed to examine for different bone grafts combination gingival derived mesenchymal stem cells (GMSCs) in bone regeneration potential application. GMSCs seeded onto the bone grafts (TCP, ProSorb , BioOss). The proliferation status of GMSCs in different scaffolds was analyzed, and the osteogenetic efficacy was evaluated after osteogenic induction. In vivo transplantation, after 6 weeks implants were harvested and paraffin section subsequently processed for H&E staining and immunohistochemical analysis. Immunohistochemical staining show that osteopontin (OPN) and type I collagen were at high levels in experimental group compared with control group. The results demonstrated that GMSCs bad the osteogenic potential in sinus floor augmentation.

Keywords: mesenchymal stem cells; gingiva; osteogenesis; bone grafts

Session No.: P3-106

Reprogramming of Mensenchymal Stem Cells into Human Induced Pluripotent Stem Cells Under Xeno-free and Feeder-free Conditions

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Human pluripotent stem cells (hPSCs), such as human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs) are an attractive prospect for tissue engineering and regenerative medicine. However, conventional hESCs and hiPSCs cultivation is limited to apply on clinics for several reasons: (a) Conventional hESCs is limited by remaining the ethical concerns, (b) hiPSCs is generated by virus transduction which is risky because the oncogene might integrate into host chromosome, (c) hPSCs are cultured on feeder layers or in other xeno-containing environments, which are also remaining unpredictable outcomes for hosts. Therefore, the development of hiPSCs under xeno-free and feeder-free conditions is a promising strategy to solve the problems for their applying to clinics. In this study, we extracted and cultivated mensenchymal stem cells such as human adipose-derived stem cells (hADSCs) or human amniotic fluid stem cells (hAFSCs) under xeno-free conditions. Sendai-virus was applied as non-gene integrated method to reprogram cells into hiPSCs. By cultivating cells under chemical-defined and xeno-free ES medium, we compared the hiPSCs generation efficiency under different conditions: (a) on feeder-free (Matrigel), (b) on human recombinant vitronectin, (c) Synthemax II dish (commercial dishes which coated with oligo-vitronectin), (d) Cellstart, and (d) different elasticities of polyvinyl alcohol-co-itaconic acid (PVA-IA) hydrogels grafted with nanosegment (oligo-vitronectin), which are another promising feeder-free culture dishes for stem cell cultivation. The results showed that the cells can be reprogramed into hiPSCs successfully under all the conditions and with higher hiPSCs generation efficiency (0.02~0.19%). The xeno-free hiPSCs are able to be maintained for more than 30 passages and remain the potential to be differentiated into different lineages that will be benefit on either clinics or drug screening as well as toxicological experiments.

Session No.: P3-107

Analysis of Senescence-related Differentiation Potential and Gene Expression Profiles in Human Dental Pulp Stem Cells

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Introduction: Dental pulp stem cells (DPSCs)-mediated dental pulp regeneration is considered as a promising method for treatment of deep caries with pulpitis, enabling conservation and restoration of teeth. However, mesenchymal stem cells (MSCs) senescence is an adverse factor for the perspective of cell-based therapies. In this study, we investigated the characteristics and expression profiles of DPSCs from young and old donors. Methods: DPSCs from young and old donors were cultured in differentiation medium and then detected their differentiation potential. Long noncoding RNA (LncRNA) microarray assays and bioinformatic analysis was performed to investigated the differences in lncRNA and mRNA expression profiles between DPSCs from young and old donors. Results: We found that DPSCs from young donors exhibited more powerful proliferation ability, osteogenic and adipogenic differentiation potentials compared with DPSCs from old donors. In DPSCs from young donors, numerous lncRNAs were significantly up-regulated (n=389) or down-regulated (n=172) compared to DPSCs from old donors. Furthermore, 304 mRNAs were differentially expressed including 247 up-regulated genes and 57 down-regulated genes in DPSCs from young donors. Bioinformatic analysis identified that several pathways may be associated with DPSCs characteristics, such as Cell cycle, RNA transport, etc, and revealed that NFYB, GTF2B and NR3C1 were identified as core regulatory factors and FR249114, FR299091 and ENST00000450004 were identified as core lncRNAs. Conclusions: Our results indicated that senescence impaired the proliferation and differentiation potentials of DPSCs, donor age is an important factor which affected their usage for tooth regeneration. We also provided insight into the mechanisms responsible for the senescence in DPSCs.

Session No.: P3-108

A Comprehensive Study of Palate Development in Miniature Pig

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Palate development is an important morphogenetic event in facial development, including the fusion of the lateral and medial nasal portions of the frontonasal process and maxilla. Abnormalities Derailments of any of these events may result in cleft palate, the most frequent congenital craniofacial abnormality. Recent research has shown that the microanatomy of the miniature pig oral maxillofacial region is quite similar to that of humans, and the use of miniature pigs as a large animal model for dental and orofacial research is increasing. Little information is available, however, about the development of the miniature pig palate. Here, using histological and ultrastructural methods, we describe the developmental stages of the palate in miniature pigs. Sections from E26, E30, E35, E40, E45, and E50 embryos were stained with hematoxylin–eosin, and selected specimens were also processed for electron microscopy. The development of the miniature pig palate can be divided into four stages: growth of the bilateral palatal shelves alongside the tongue at E30; elevation of the horizontal position above the tongue at E35; establishment of bilateral shelf contact at the midline from E35–50; and a final fusion step at E50, similar to mouse and human. The histological characteristics of the miniature pig palate at different developmental stages were synchronously verified at the ultrastructural level. Our study provides first-hand data regarding palate morphological organogenesis in the miniature pig and a foundation for further research with this model to explore mechanisms of cleft palate development.

Session No.: P3-109

Application of Epigallocatechin-3-gallate on Stretch-induced Osteogenic Differentiation of Human Mesenchymal Stem Cells

Ji Won Shin[1], Yun Gyeong Kang[1], Yanru Wu[1], Hun Young Ban[1], Seo Rin Gu[1], Jung Hyun Choi[2], Hyun Ju Choi[2], Ji Eun Kim[1], Jung-Woog Shin[3]

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Epigal¬locatechin 3 gallate (EGCG) has been reported to prevent various diseases, including osteoporosis. Osteogenic differentiation of mesenchymal stem cells (MSCs) plays an important role in osteoporosis. And mechanical stretching is one of the most important physical stimuli for osteogenic differentiation. Despite advances in stem cell biology, there are few bioactive compounds tested in relation to the osteogenic differentiation of human mesenchymal stem cells. In the present study, we tried to compare osteogenic potential of MSCs under cyclic stretch of 3% elongation at 0.2 Hz on 4 consecutive days, 4hrs/day in combination with or without EGCG. Cyclic stretch induced a significant expression of runt-related transcription factor 2 (RUNX2) and EGCG also had a synergistic effect on that of RUNX2. Gene expression analysis revealed that EGCG also had effect on mitochondrial antioxidant (SOD1, SOD2, CAT) and biogenesis (PGC-1alpha, TFA) upregulation in stretch-induced osteogenic differentiation. These upregulations may be induced by VEGF and BMP2, both of which were affected and upregulated by EGCG with stretching. However, VEGF were not significantly affected by EGCG treatment alone. These result indicated that treatment EGCG itself was able to enhance osteogenesis. In this study, we demonstrated that EGCG exerts a significantly positive effect on stretch-induced osteogenesis and treatment with EGCG was dependent on the stretch which is osteogenic inducer of hMSCs. In conclusion, EGCG have a great potential as a therapeutic agent in osteoporosis but more studies should be done to confirm its safety and mechanism. Acknowledgement: This work was supported by the Human Resource Training Program for Regional Innovation and Creativity through the Ministry of Education and National Research Foundation of Korea(NRF-2014H1C1A1073148) and by the National Research Foundation of Korea (NRF) Grant (NRF-2014K2A2A7066637).

Session No.: P3-110

Chromatin Remodeling in a Nucleus During the Differentiation of Human iPSc into Cardiomyocytes by Texture Analysis

So Hee Park[1], Young Mi Kim[1], Yun Gyeong Kang[1], Seo Rin Gu[1], Yan Ru Wu[1], Hun Yeong Ban[1], Mi Jin Kim[1], Eun Jin Lee[1], Jung-Woog Shin[2]

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Human induced pluripotent stem cells (iPSc) are known to be differentiated into any type of cell, even into cardiomyocytes under proper conditions. It is also widely recognized that chromatin remodeling inside a nucleus occurs during differentiation. Therefore, it could be the first step to investigate chromatin remodeling for the understanding of differentiation mechanism inside the nucleus. In this study we tried to investigate chromatin remodeling during the differentiation of iPSc into cardiomyocytes. For the quantitative investigation we adopted GLCM (Gray-Level Co-occurrence Matrix) analysis, one of the texture analysis techniques, based on matlab. Human iPSc were seeded onto matrigel-coated plates at a density of 100000 cells/cm2 in ES culture medium containing 4 ng/mL of bFGF for 5 days before induction. To induce differentiation, we replaced by RPMI+B27 medium supplemented with 100 ng/mL of Activin A for 1 day, followed by 10 ng/mL hBMP4 for 4 days. After 5 days, the medium was then exchanged for RPMI+B27 without any supplementary cytokines. The iPSc marker (SSEA-4) and cardiomyocyte marker (GATA-4) were confirmed by immunofluorescence staining to determine they were differentiated or not. Chromatins in a cell nucleus were stained using 4', 6-diamidino-2-phenylindole. The energy indicating chromatin remodeling was calculated utilizing GLCM algorithm for texture analysis. The analyses showed that it tended to decrease in energy level as differentiation went on. It can be suggested that chromatins were being condensed when differentiation was going on. Consequently the lower energy level was observed and calculated when differentiation was almost completed. From this study we could confirm the chromatin condensation during differentiation and this quantitative evaluation utilizing GLCM could be another indicator showing degree of differentiation. This work was supported by the National Research Foundation of Korea (NRF) Grants (NRF-2013R1A1A2059219, NRF-2012M3A9C6050370).

Session No.: P3-111

Significant Increase in iPSC Formation Due to Mechanical Stimuli and Its Potential Cues

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Since the first report on induced pluripotent stem cells(iPSc) generation was released, researchers have suggested numerous methods to improve reprogramming efficiency. Apart from safety concerns, the current reprogramming methods still have some limitations including a low efficiency and slow kinetics during reprogramming process. Moreover, the role of mechanical stimuli during reprogramming has not been studied. In this study, we demonstrated the potential of tensile stimulation in improving efficiency and in reprogramming process. Additionally, we could confirm certain cytokines secreted by tensile stimuli. The human dermal fibroblasts(hDFs) were seeded onto both general 6-well plates and 6-well BioFlex plates at day 1. At day 2 retroviral supernatant from each of four reprogramming factors(Oct3/4, Sox2, Klf4, and c-Myc). From day 4 to 7 equi-axial cyclic stretching of 8% was applied to flexible well membranes for 2 hours with 25 sec and 125 sec for stretching and resting, respectively. We found significant increase of efficiency in reprogramming and iPSc generation. For the reasoning of this increase we tried to use conditioned medium acquired during stimulation. The medium was acquired right after 24 hours' stimulation, and it was used for the reprogramming of the hDFs which contained viruses. This process was done 4 days in a row. Finally the transduced hDFs were re-plated onto feeder cells. Here, we demonstrated that mechanical stimuli with OSKM could promote the reprogramming of hDF cells to pluripotent state. Additionally, the secreted cytokine, even not identified, by mechanical stimuli seemed to play a role in improving reprogramming efficiency of iPSc. We hypothesized that mechanical stimuli may affect the reprogramming process and iPS cells production in human somatic cells. From these preliminary results biophysical effects on cell reprogramming, especially on iPSc formation are worth to be investigated. This work was supported by the National Research Foundation of Korea(NRF) Grant(NRF-2012M3A9C6050370, NRF-2015M3A9B6073642).

Session No.: P3-112

The Effect of Knockdown of Spry4 in Human Adipose-derived Stem Cells Differentiation

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The Sprouty4 (Spry4) gene is binding to RAS binding site on RAF1, thereby inhibiting of the RAS-MAPK signaling pathway. It is involved in growth factor mediated signaling pathway on stem cells. The proliferation and differentiation of stem cells are regulated through the Erk1/2 phosphorylation which the output of MAPK signaling pathway. In the present study, we revealed the effect of Spry4 on differentiation of human adipose-derived stem cells (hASCs). For this study, we knock down the Spry4 gene of hASCs using siRNA. Each differentiation was measured by quantitative RT-PCR, western blotting, and histological analysis. In osteogenic differentiation, the osteogenic marker genes and proteins expression were significantly increased in siSpry4-treated hASCs than siControl-treated hASCs which used for control group. Even on the staining of mineralization also shown that siSpry4-treated hASCs have increased osteogenic differentiation efficiency as compared to the control. In chondrogenic differentiation, the chondrogenic marker genes expression was no difference between siSpry4-treated hASCs and control. However, the chondrogenic marker proteins expression was decreased in siSpry4-treated hASCs compared with control.even on staining of glycosaminoglycan (GAG) also shown that siSpry4-treated hASCs decreased as compared to control. In addition, hypertrophic marker genes expression was increased in siSpry4-treated hASCs while chondrogenic differentiation. In adipogenic differentiation, although PPARγ expression was increased in siSpry4-treated hASCs as compared to control, there was no difference in Adiponectin and C/EBPβ gene expression of siSpry4-treated hASCs as compared to control. likewise, the siSpry4-treated hASCs have no difference as compared to control on lipid drop staing. As a result, the knockdown of Spry4 caused increase in ERK 1/2 phosphorylation as MAPK signaling significantly enhances the efficiency of osteogenic differentiation. However, the chondrogenic differentiation is decreased and the adipogenic differentiation is not affected by the knockdown of Spry4 in hASCs.

Session No.: P3-113

Image-based Profiling of Chemical Compounds for Regulating Stem Cell Culture

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By the rapid advances in stem cell applications, various types of culture protocols have been developed for stem cell culture. For such new stem cell culture protocols, small chemical compounds are screened as candidate molecule to regulate the functions of stem cells to replace costly protein additives. To eliminate the risk of unexpected infectious risks and quality instability of animal-derived crude materials as medium additives, such chemically defined molecules that can be produced and tested with strict quality check is required strongly for stem cell applications such as cell therapies. Some reports also have indicated that such differentiation controlling compounds can be used as medical drug seed molecules. However, the conventional cell assay technologies had been facing a great difficulty. Since stem cell culture requires long in vitro culture, conventional molecular-biological evaluation techniques have been found to be limited to assay end-point reactions because of their invasive manner. To acquire more delicate and continuous cellular responses to small molecule additives, we have invented non-labeling image-based morphological analysis technique by combining image processing and bioinformatic algorithms. We here show the profiling of 6 chemical compounds, inhibitors of gene network toward cytoskeleton activation, for the regulation of adipogenic differentiation. By our method, we succeeded in screening “enhancers” only from the very early stage of non-stained cellular morphologies.

Session No.: P3-114

Nitric Oxide Promotes Osteogenic Differentiation of Adipose Derived Stem Cells Through Activation of the Wnt/-β-Catenin Signalling Pathway

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Nitric oxide (NO) is a diffusible gas which has a wide variety of pleiotropic effects including stem cell differentiation. Endothelial nitric oxide synthase (eNOS), is one of the three enzymes responsible for producing NO, and is located at the plasma membrane in a tight conformation with the caveolin 1 (CAV-1) protein. This interaction between eNOS and CAV-1 results in low NO production. In this study, we aimed to increase NO production by genetically modifying equine adipose derived stem cells (EADSCs) with eNOS and a non-inhibitory Cav-1 mutant (CAVF92A) and to assess Wnt signaling in relation to osteogenic differentiation.

Nitrite as an indirect measure of NO was significantly increased in eNOS and CAVF92A transduced EADSCs (5.59 ± 0.22 μM) compared to eNOS alone (4.81 ± 0.59 μM) and non transduced control cells (0.91± 0.23 μM) ($p<0.05$). During osteogenic differentiation , higher NO correlated with increased alizarin red staining, upregulation of Runx2 and alkaline phosphatase (ALP) gene expression and the activity of a novel Runx2 promoter driven GFP reporter compared to eNOS alone transduced cells and non-transduced controls.

Canonical pathway associated Wnt3A and Wnt8a gene expression was increased in eNOS-CAVF92A cells undergoing osteogenesis whilst non-canonical Wnt5a was decreased. To further analyse the role of NO and the Wnt/-β-catenin pathway during osteogenesis , EADSCs were co-transduced with a lentiviral TCF/LEF-GFP reporter system and a doxycycline inducible eNOS vector, GFP mRNA transcript levels were up-regulated only when eNOS was activated by addition of doxycycline,. Finally lentiviral vector expression of Wnt3A in EADSCs was also associated with enhanced osteogenesis as was treatment of cells with an exogenous NO donor (NONOoate), which resulted in similar upregulation of Runx2 and ALP. In summary, manipulation of nitric oxide levels can modulate the Wnt/-β-catenin signaling pathway to enhance osteogenesis in EADSCs which may contribute novel bone regeneration strategies

Session No.: P3-115

Integration of Mesenchymal Stem Cells and Mechanical Stimuli in Hematopoietic Stem Cell Expansion and Maintenance

Yun Gyeong Kang[1], Seo Rin Gu[1], Ji Won Shin[1], So Hee Park[1], Young Mi Kim[1], Yanru Wu[1], Mi Jin Kim[1], Hun Yeong Ban[1], Eun Jin Lee[1], Jung-Woog Shin[2]

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Hematopoietic stem cell(HSC) transplantation is used in the treatment of blood-related malignant and/or inherited diseases. Ex vivo expansion of HSCs has been investigated to improve the clinical outcome of HSC transplantation, particularly in resolving limited grafting. Expansion of HSCs contains major problem that proliferating cells tend to differentiate easily, presumably due to the lack of cues provided by the in vivo microenvironment. In nature, HSCs are located mainly in the bone marrow where they interact within a HSC niche, which regulates HSC fate. This study was performed for ex vivo expansion of HSCs co-cultured with mesenchymal stem cells(MSCs). The analyses were separately performed on HSCs whether they were direct-contacted or non-contacted with MSCs. Moreover, to investigate the effect of the mechanical environments, various hydrostatic pressure(HP) were adopted when co-cultured with MSC. 20kPa HP was found effective in expansion of total nucleated cells, HSC and primitive HSC, when HSCs were co-cultured with MSCs. Furthermore, the direct-contact fraction revealed less-differentiated phenotypes than those of HSCs in the non-contact fraction. MSCs co-cultured with HSCs expressed substantially higher levels of HSC niche markers compared with MSCs alone. Especially, the application of 20kPa HP induced significantly higher levels of all markers compared with other HP magnitudes. The results can be summarized as: 1)mechanical stimuli can modulate the expression of specific genes, 2)HP(20kPa) is effective for HSC expansion and maintenance, and 3)HP has synergistic effects with cell-to-cell interaction. Namely, this biomimetic experimental study presented that applying the optimal magnitude of HP and direct-contact co-culture with cells consisting of HSC niche enhanced HSCs expansion and maintenance. This study was the first to apply HP in expansion of HSCs. These results are expected to have an important impact on the design of a clinical-scale expansion system. [This work was supported by the NRF of Korea Grant (NRF-2015M3A9B6073642).]

Session No.: P3-116

Tracheal Motion Bioreactor: A Dynamic Culture System for Tracheal Cartilage Tissue Engineering

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Tissue engineering an ideal tracheal substitute with rigid support is required for improving tracheal reconstruction. In previous study, we cultured chondrocytes into Poly(ε-caprolactone) (PCL) scaffold under static environment and successfully constructed a cartilage ring in vivo with adequate chondrogenesis. However, improvement of cell-seeding method and acceleration of chondrogenesis in vitro are still challenges in tracheal tissue engineering. Dynamic environment may play an important role in maturation of some special tissue. Recent studies had focused on tissue engineering assisted by bioreactor and confirmed that a dynamic environment can promote tissue regeneration. Thus, we developed a bioreactor to mimic the dynamic environment of tracheal motion during eupnea, and tested the effects of this tracheal motion bioreactor on cartilage formation in PCL scaffold in vitro. We utilized a hydraulic pressure system in the bioreactor to generate the radial expansion force toward the cells and scaffolds. The bioreactor was monitored during the test at constant pressure of 1.5 psi and frequency of 32 times/min for mimicking tracheal motion of human. No effect of bioreactor on appearance of PCL scaffolds was observed by scanning electron microscope after a 7-day incubation, suggesting the bioreactor did not affect the structure of PCL scaffolds. In contrast, Micro-CT analysis showed that bioreactor induced chondrocytes infiltration in the inner space of PCL scaffolds. This finding was subsequently confirmed by H&E staining. Similarly, the level of glycosaminoglycan was significantly increased in the group with bioreactor, suggesting the bioreactor also induces the expression of extracellular matrix. Together, our results indicate that tracheal motion is critical for in vitro chondrogenesis, including the chondrocytes infiltration and extracellular matrix expression. We also provide a novel dynamic culture system which can be applied to improve the tracheal tissue engineering.

Session No.: P3-117

No Modulated the Effects of L-theanine on the Stress-induced Oxidative Stress and Neuroinflammatory in Rat Hippocampus

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Stress elevates the pathophysiological activities resulting in disrupted homeostasis. Among experimentally stressed rats, the production of lipid peroxidation (LPO) byproducts, inflammatory cytokines and nitric oxide (NO) were increased in the brain; these increased oxidative stress, inflammatory cytokines and NO were correlated with behavioural impairment. L-theanine (L-T), an amino acid component of green tea, is able to scavenge reactive radicals and decrease peroxidative reactions, as well as has neuro-protective effects against ischemia, toxin and stress-induced behavioral impairments. In this study, we examined the potential protective properties of L-theanine and its implication with the NO mechanism in reducing the stress-induced oxidative stress and neuroinflammatory in rat hippocampus.

Session No.: P3-241

The Development and Application of Therapeutics for Neurodegenerative Diseases

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As global populations age, the prevalence of neurodegenerative diseases for the central nervous system (CNS) has increased significantly. Although huge efforts have been put into basic research, target validation and clinical trials, there is still no effective medicine for neurodegenerative diseases. Development Center for Biotechnology(DCB) has years of experience in developing biologic medicines from target identification, lead optimization to pre-investigational new drug(pre-IND) application. DCB also carries many patents in the manufacturing process and the biologics product. Approaches to transfer biologics across the blood-brain barrier are under development. Studies had shown that neuroinflammation is involved in various neurodegenerative diseases, such as Alzheimer’s disease and Parkinson’s diseases. Chronic neuroinflammation may induce neuronal death and cause the degradation of brain tissue. Our focus is developing therapeutic biologics to reduce chronic neuroinflammation or to protect neurons from degeneration. While generating multiple drug hits and leads, distinct functional assays and animal models of diseases are set up to screen for the candidate drugs. DCB is building a center of excellence (CoE) to promote and share our capability, and to collaborate with any other research peoples/teams. DCB's mission is to facilitate the development of Taiwan's biotechnology industry by building the infrastructures, developing key biotechnologies, and training and recruiting professional workforces, in coordination with industrial, governmental, academic, and research institutions.

Session No.: P4-118

Cadherin-functionalized Biodegradable Polyurethanes Promote Tissue Specificity from Human MSCs via Modulation of Canonical Wnt Signalling

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Cell-cell interactions mediated by cell adhesion molecules, E-and N-cadherin, play important roles in cartilage and bone development; however their role in regenerating osteo-chondral tissues that are lost by trauma or disease is unclear. Degradable polyurethanes (dPUs) have been widely used for biomedical applications but have intrinsic limited potential for cartilage or bone regeneration due to their lack of conductive or inductive cues. The aim of this study was to develop a novel dPU polymer modified with cadherin mimetic peptides and to thereafter investigate the effect of these modified polymers on osteo/chondrogenic differentiation of human mesenchymal stem cells (hMSCs) in vitro and in a rat femur head defect model in vivo. In this study, we synthesised azide-functionalised dPU polymers (dPU-N3) by converse of the terminal hydroxyl group into tosylate. Using thermally induced phase separation (TIPS), 3D porous dPU-N3 scaffolds were fabricated and thereafter post-functionalized with E-cadherin (CDH1) or N-cadherin (CDH2) mimetic peptides using click chemistry. hMSCs were employed for in vitro osteo/chondro differentiation on dPU-CDH TIPS scaffolds, and scaffolds with or without hMSCs transplanted into a rat femur bone defect model for in vivo assessments. In vitro experiments showed that dPU-CDH1 thin films and scaffolds significantly increased mineralization and osteogenic markers ALP, OCN, osteopontin (OPN), while dPU-CDH2 thin films and scaffolds conversely increased chondrogenic markers, aggrecan, Sox9 and COL10A1, when compared to that of dPU-N3 only. Our in vivo data showed that transplantation of hMSC-seeded dPU-CDH1/2 polymer scaffolds resulted in significant differences in new bone/cartilage tissue formation (confirmed by changes in bone/cartilage markers ALP, OCN, aggrecan, COL10A1) and Wnt-related molecules (WNT3a, AXIN2 and β-catenin), when compared to that of dPU-N3 control group. Our data suggest that modification of a dPU-polymer with cadherin mimetic peptides can enhance and drive the regenerative capacity of stem cells for applications in bone/cartilage tissue engineering.

Session No.: P4-119

Bioengineering of Hepatic Microtissues by Scaffold-free 3D Cell Assembly

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Liver is the most significant organ in drug metabolism in human body. In native liver tissues, hepatocytes are made up of 70-85% liver's wet mass. High hepatocyte packing density and close cell proximity are crucial to maintain hepatocyte viability and metabolism functions. Conventional scaffold-based tissue engineering methods and emerging bottom-up microgel assembly methods can't achieve high cell packing density for liver tissue engineering. Herein, we demonstrate a unique scaffold-free 3D cell assembly approach to bioengineer 3D hepatic microtissues. We explored Faraday wave patterned hydrodynamic drag field to assemble a large amount (up to 5 million) of primary hepatocytes into a predefined 3D cellular construct in a liquid container in around 10 seconds. The geometry of the cellular construct can be dynamically tuned via frequency and amplitude of Faraday waves. After cell assembly, the cellular construct was immobilized in fibrin hydrogel for further tissue culture. Closely packed hepatocytes formed hepatic microtissues within three days with evidences of formation of bile canaliculi validated by phase contrast microscopy and CMFDA assays. Immunofluorescence showed expression of hepatocyte specific markers including albumin and multidrug resistance protein 2 (MRP2) in day 7. ELISA analysis of culture medium also indicated continuous secretion of albumin from hepatocytes into surrounding microenvironment over the 10-day tissue culture. In sum, our method provides a unique approach to bioengineer tissues with high cell density and low extracellular matrix and potentially facilitates wide application in tissue engineering and regenerative medicine.

Session No.: P4-120

Osteoimmunomodulation for the Development of High-performance Bioceramic Coatings with Functional Osseointegration

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A number of coating materials have been developed seeking to improve the osseointegration of orthopaedic implants. Despite many candidate materials trialled, the low successful clinical translational rate indicates that current strategies for their development could be improved. The paradigm on the nature of biomaterials has shifted from being an ‘inert’ material to ‘immunomodulatory’, indicating the importance of immune reaction in the host/biomaterials interactions. Specifically in bone environment, osteoimmunology indicates that the close relationship between the immune cells and bone cells, suggesting the rationale of using biomaterials to manipulate the immune response thereby enhancing osseointegration, which is defined as osteoimmunomodulation. Osteoimmunomodulation may become a novel strategy for the development of high-performance bioceramic coatings. Nutrient elements, such as Sr, Mg, Zn, Si, have been known to elicit significant effects on regulating immune response. Therefore, we tried to combine nutrient elements into novel types of coating materials, aiming at manipulating osteoimmunomodulation to enhance osseointegration. Three novel types of coating materials, Sr2MgSi2O7, Sr2ZnSi2O7, and MgSiO3 were successfully developed by a plasma-spray coating method. They were found to have higher bonding strength with Ti-6Al-4V substrate, compared with that of hydroxyapatite coatings. It is also found that they could release functional nutrient ions, including Sr, Mg, Si, and Zn, inducing an immunomodulation more conducive for osseointegration, demonstrated by downregurelating pro-inflammatory cytokines, enhancing osteogenesis, and inhibiting osteoclastogenesis. The MgSiO3 coated implants were implanted into the shaft of rabbit femur to evaluate the in vivo osseointegration and loading capacity. MgSiO3 coatings had vastly improved osseointegration with host bone, as shown by significantly enhanced biomechanical strength and increased de novo bone formation. These results indicate that manipulating osteoimmunomodulation using nutrient elements can be a promising strategy in developing advanced functional coating materials, which are highly potential to be translated into useful medical devices for orthopaedic and dental clinical applications.

Session No.: P4-121

Next Generation of Periodontal Regeneration Therapy: The Canonical Wnt Signalling Activator?

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Lithium (Li) has been widely used as a long-term mood stabilizer in the treatment of bipolar and depressive disorders. Li+ ions are thought to enhance the remyelination of peripheral nerves and also stimulate the proliferation of neural progenitor cells and retinoblastoma cells via activation of the Wnt/β-catenin signalling pathway. Until now there have been no studies reporting the biological effects of released Li+ in bioactive materials on cemetogenesis in periodontal tissue engineering applications. In this study, firstly we investigated the effect of LiCl injection in periodontal defect rat model. Then we incorporated parts of Li+ ions into the mesoporous bioactive glass (MBG) scaffolds. Then, this study further investigated the interactions of human periodontal ligament cells (hPDLCs) with both Li-MBG scaffolds, and further explored the osteogenic and cementogenic stimulation of these Li-containing biomaterials and the possible molecular mechanisms. Histological staining showed that the LiCl injection groups had a significant increase of new cementum formation with cementocytes entrapped and well-orientated Sharpey's collagen fibers inserted, whereas the control groups showed no cementum formation. Furthermore, we successfully yielded scaffolds with a favourable composition, microstructure and mesopore properties. The results showed that 5% Li+ ions incorporated into MBG scaffolds enhanced the proliferation and cementogenic differentiation of hPDLCs on scaffolds, most likely via activation of Wnt/β-catenin signalling pathway. Further study demonstrated that Li+ ions by themselves significantly enhanced the proliferation, differentiation and cementogenic gene expression of PDLCs. Our results suggest that the combination of Li with bioactive scaffolds may be a promising method to enhance bone/cementum regeneration as Li-containing biomaterials possess excellent in vitro osteogenic and cementogenic stimulation properties by inducing bone/cementum-related gene expression through enhancing the Wnt canonical signalling pathway to stimulate cementogenic differentiation of PDLCs.

Session No.: P4-122

Adipokines Can Promote the Proliferation of Adipose-derived Stem Cells in Vitro

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Adipose-derived stem cells(ADSC), with advantages such as large storage and easy access, has become one of the ideal seed cells for tissue engineering and regenerative medicine. Adipose tissue(AT),as an endocrinal organ which secretes numerous proteins called adipokines, is gradually drawing attention in the field of developmental biology. However, few studies have explored the effect of AT on the growth and proliferation of ADSC. This study tries to imitate the whole paracrine environment of AT by using co-culture technique, and explore such effect. The research team first cultured AT and ADSC with basal medium(DMEM supplemented with 1% FBS and 1% antibiotics) to get AT supernatant and ADSC supernatant. Then cultured ADSC with basal medium(group A),half ADSC supernatant in basal medium(group B) and half AT supernatant in basal medium(group C), respectively. The proliferation of ADSC was observed on the second, fourth and sixth day, with the method of Cell Counting Kit-8, and drawn the proliferation curve. It was found that group B had significantly higher proliferation curve than the other groups. It demonstrates that the paracrine effect of AT is able to markedly promote the growth of ADSC, and thus provides a new method to efficiently culture seed cells.

Session No.: P4-123

Rapid Prototyping of Microwells for the Formation of Cell Spheroids and the Study of Cell-ECM Interaction

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It is well accepted that for the accurate modeling of natural tissue in a laboratory setting, it is important to recreate the cells organized into a three-dimensional geometry that mimics their native environment. A common approach to form natural structures similar to mammalian tissue is to force cells to organize into globular aggregates referred to as spheroids or embryoid bodies. In order to create such structures, different techniques that force the cells to associate with each other while restricting the size of the spheroids have been used. As well as hanging-drop, non-adherent surfaces microwells have been shown to be particularly efficient while being compatible with the usual biological characterization assays. To date, many studies have focused on replicating and characterizing the interactions taking place inside the spheroids, such as cell-cell junctions and communication. However, in order to provide a better understanding of spheroid dissociation, growth and survival, systems that provide a convenient way to reproduce the mechanical and biological interactions between the spheroid and the surrounding ECM are required. In order to engineer microwells that could mimic the natural cell environment access to high precision and expensive manufacturing tools is often required. Once prepared, these microwells platform cannot be easily functionalized nor modified to incorporate biological signaling that could trigger the association, migration or guide the function of the cells and to spheroid. Herein we present the development of a polydimethylsiloxane based microwell system. Through rapid and cost effective prototyping, the well dimensions and shape are tailored to fit the targeted spheroid size, and incorporation of site-isolated specific surface chemistry enables the fast and easy tethering of biological signals. These features enable the rapid screening of the impact of the well size, well shape and the immobilized biomacromolecules on the spheroid survival, function and dissociation.

Session No.: P4-124

Ex Vivo Cultivation Model to Evaluate Fish Scale as a Potential Cell Transplantation Carrier: Effect of Extracellular Matrix Coating on the Growth and Function of Corneal Endothelial Cells

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To investigate the feasibility of using fish scale as a cell transplantation carrier, its modification, and underlying mechanism, decalcified fish scales of Taiwan Tilapia were coated with fibronectin, laminin, collagen IV, or FNC® coating mix (FNC) with or without further cross-linking with EDC/NHS. Attachment and proliferation of human corneal endothelial cell line B4G12 cells cultured on the scales were evaluated. cDNA microarray was used to compare gene expression profile in B4G12 cells cultured on FNC-coated fish scale. Surface coating with extracellular matrix (ECM) proteins significantly increased the attachment and proliferation of B4G12 cells on fish scale, which expressed normal endothelial cell differentiation markers ZO-1 and NA+-K+ ATPase. Cross-linking of ECM proteins to fish scales did not affect the attachment-promoting effect of ECM. Results from cDNA microarray, Q-PCR, and Western blot showed that ECM coating may: 1. Up-regulate integrin signaling pathway, which in turn up-regulates the Wnt pathway, facilitating cell proliferation. 2. Up-regulate enzymes associated with sugar metabolism, so that the cells are more metabolic active. 3. Up-regulate carbonic anhydrase 12, a fluid transport-associated enzyme. Silencing ILK expression by siRNA may down-regulate CA12 expression, suggesting that transcription control of CA12 is under the influence of ILK pathway. By surface coating with ECM proteins, we have demonstrated that fish scale is capable of being used as a culture substrate and carrier for corneal endothelial cells. By activating integrin pathway, surface coating promotes the attachment and proliferation of B4G12 cells. Integrin pathway may also up-regulate CA12 expression to facilitate fluid transport in corneal endothelial cells. Furthermore, ECM coating may up-regulate enzymes associated with sugar metabolism to increase metabolic activity of the cells. This research provides a novel cell carrier and its modification strategy. Hopefully this can promote the realization of cell therapy using cultivated human corneal endothelial cells.

Session No.: P4-125

Evaluation of the Culture Process of Human Autologous Corneal Epithelial Sheet

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In regenerative medicine utilizing autologous cells, cultured cells are collected from a patient, constructed a tissue, and transplanted back into that patient to treat. In this study, we focused on autologous cultured corneal epithelial cell sheet used in transplantation therapy for refractory corneal epithelial disease. We evaluated the heterogeneity of the corneal epithelial cells during its culture process by observing the cell behavior and sheet formation. Method: Human autologous limbal stem cells were co-cultured with 3T3-J2 feeder cells then be used to produce cell sheet. We evaluated the differences between samples based on the distribution of cell density and epithelial stem cells in the basal layer from images obtained by confocal laser scanning microscopy. Here we stained the cells using p63 and Hoechst 33258 as the epithelial stem cell marker and nuclear marker, respectively. Moreover, we acquired the time-lapse image (every 20 minutes for 15 days) using the non-invasive phase-contrast microscope. Results: Comparing three-dimensional stained images and cell dynamic behavior data, we suggest there is a correlation between the distributions of epithelial stem cells in the basal layer and the cell motility after stratification. Specifically, in the sample where cells stopped migration after stratification even after 9 days, many p63 positively expressed nuclei in the basal layer were present as a group. In contrast, in the sample where cells continued migration after stratification, it was observed that the cell density decreased with the cultivation process, and the p63 positively and negatively expressed nuclei were mixed in the basal layer. Conclusion: The non-invasive observation of cell dynamic behavior was suggested to be an index for the quality assessment of cultured corneal epithelium sheet.

Session No.: P4-126

Structural Studies of Human Glycosylated VEGF-A121 and VEGF-A165 Provide Mechanistic Insights into the Restricted Approach of Neuropilin in Heparin-Mediated Stabilization of the Ternary Complex

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A bivalent sugar moiety with N-linked glycosylation in receptor-binding domains of glycosylated VEGF-A121 dimer is described through the determination of crystal structure to a resolution of 1.71 Å. This precisely placed sugar structure ensures the structural accuracy for the atomic constructions of a SAXS-envelope for the full length VEGF-A165. Such a complete VEGF structure provides evidence for the restricted approach of heparin and co-receptor neuropilin from the membrane side, excluding the possibility as previously anticipated also from the receptor-binding side. In addition, based on the deduced bending required for connecting the heparan sulfate chains with sufficient monosaccharide units when considering both heparin binding pockets on VEGF-A165 and neuropilin are fully occupied, the previously illustrated “near-quantitative conversion” of neuropilin in forming the ligand/co-receptor complex thereby to stabilize the receptor dimer can be examined in structural aspects.

Session No.: P4-127

Reconstitution of Nucleus Pulposus Cells Matrix Niche in 3D Collagen Microspheres

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Background: Recently, attempts to treat disc degenerated disease (DDD) have turned to seeking answers from tissue engineering and regenerative medicine. A possible approach is to differentiate cells exhibiting plasticity such as mesenchymal stem cells (MSCs) and dermal fibroblasts (DFs) into the disc-like cells by exposing them to a biomimetic microenvironment in the disc. However, there is no systematic study in identifying and optimizing the biomimetic microenvironment, which is able to alter the phenotype and the fate of the plastic cells toward discogenic lineage. Methods: In this project, we attempt to reconstitute the biomimetic matrix microenvironment of the native nucleus pulposus (NP) tissue. Our approach was to culture nucleus pulposus cells (NPCs) within collagen microspheres whilst maintaining their phenotype and other characteristics, so that they would remodel the matrix microenvironment. Upon removal of the original NPCs, the reconstituted NPC-derived complex matrix was evaluated by proteomic analysis. Results: Previously we demonstrated that NPCs could maintain survival within the collagen microspheres and produce NP-like ECM such as glycosaminoglycan (GAG) and Type II Collagen. According to the Mass Spectrometry (MS) results, an acellular matrix with partial retention of the ECM was obtained after decellularization. The decellularized microspheres were able to be repopulated with human MSCs and DFs. Conclusion: A complex composition of the reconstituted acellular NPC matrix niche was able to be partial preserved and this acellular matrix presented a potential system for inducing plastic cells differentiation into the NPC-like lineage. Ongoing efforts, include real-time PCR, will be use to assess the effects of the acellular matrices on plastic cells fate.

Session No.: P4-128

Surface Modification of Poly(Dimethylsiloxane) (PDMS) to Prolong Cell Culture Analysis on a PDMS Based Substrate

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Poly(dimethylsiloxane) (PDMS) is extensively exploited to study cell physiology in the field of mechanobiology and microfluidic chips due to their numerous advantages over several other materials which include transparency, low cost and ease of fabrication. However, the intrinsic high hydrophobicity of PDMS render an incompatible surface for stable cell adhesion and proliferation. While plasma treated or protein coated PDMS can improve the surface properties, these strategies are often short-lived, and accompanied with cell aggregates formation upon cell confluence. Thus, this has limited the use of PDMS microfluidic chip in the long term analysis of cell physiology. In order to tailor a biocompatible surfaces for long term cell culture, we explore different surface modification to the PDMS surfaces, and investigate its effects on the cell viability. In our studies, we adopted physical, chemical or biological modification strategies and assessed their efficiency in prolonging mesenchymal stem cell for a period up to 3 weeks. Characterization of these modified PDMS surfaces revealed a significant reduced in hydrophobicity when compared to non modified surfaces. Following that, cellular analysis revealed much stability in cell adhesion and the multipotency of the mesenchymal stem cells was well maintained. Hereby, we establish several feasible method to enhance the biocompatibility of PDMS as a potential tool for long term cell analysis in either PDMS-based microfluidic technologies or mechanobiological studies.

Session No.: P4-129

The Interplay of Cellular/Matrix Alignment and Growth Factor on Mesenchymal Stem Cells-based Cell Sheets Engineering for Later Multi-lineage Differentiation

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Integrating mesenchymal stem cells (MSCs) and cell sheet technology (CST) has expanded the potential of CST in generating two and three-dimensional tissue constructs of suitable cell types for transplants. However, current cell sheet engineering in vitro does not resemble a microenvironment in the native tissues such as the presence of growth factors and matrix alignment. The lack of these stimuli may direct stem cell based cell sheet into an undesired lineage in the later tissue reconstruction. In this study, we examine the interplay effect of aligned microtopography and growth factors on stem cells based cell sheet formation and how these pre-conditioned cell sheet could influenced later differentiation. Our study first focused on the effect of varying micro-grill width on cells where cellular alignment is pivotal, such as those in the tendons and in the Annulus Fibrosus (AF). Following that, we investigate how growth factor could influence the formation of aligned MSCs-based cell sheet and access the potential of these pre-conditioned cell sheet in the later tissue formation of tendons and AF in vitro. Our results showed that culturing MSC on 15μm gap width induced alignment in the cell sheet, upregulated ECM production and upregulated tendon-specific and fibrocartilage-specific markers during cell sheet formation. In addition, different growth factor supplementation also resulted in different expression profile for tendon and fibrocartilage markers. We further showed that pre-conditioned aligned cell sheet has better differentiating capability as compared to non-conditioned aligned cell sheet. Thus, our findings allow insights into determining the combination of biochemical and physical factors in tailoring MSC-based cell sheet engineering for future aligned tissues regeneration.

Session No.: P4-130

The Facilitating Effects of Adipose-derived Stem Cell on Tumor Initiation and Growth Assisted by Bioluminescence Imaging

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Adipose-derived stem cells (ADSCs) are multipotent cells that have attracted much recent attention and emerged as therapeutic approaches in several medical fields. However, the elusive role of ADSCs in tumor development causes the safety concerns in clinical utilization. In this study, primary ADSCs were isolated from the abdominal fat of mice to investigate their impacts on tumor development of breast and colon cancers. The in vivo animal model of tumor formation is monitored by bioluminescence imaging. We demonstrated that ADSCs enhance sphere formation and in vivo tumor initiation of breast and colon cancer cells. Parallel to the tumor initiation results, upon co-culture with ADSCs cancer cells revealed upregulation of several cancer stem cell markers, including SOX2, NANOG, ABCG2, and ALDH1A1. ADSCs also accelerated growth of breast and colon cancer cells in vitro and in vivo and raised the expression of MKI67 and PCNA proliferation markers in cancer cells. Furthermore, we determined that ADSC interaction with cancer cells stimulates increased secretion of interlukin-6 mainly from ADSCs, which then acts in a paracrine manner on cancer cells to enhance their malignant properties. Interleukin-6 was further identified to regulate genes related to cancer stem cell and cell proliferation and to activate JAK2/STAT3, a predominant interleukin-6-activated pathway, in cancer cells. Collectively, we demonstrated that ADSCs play a pro-malignant role in tumor development of breast and colon cancer cells through activation of interleukin-6-related pathway. The current study indicated that ADSCs enhance the tumor initiation and formation of breast and colon cancers, which is important for safety considerations regarding the clinical application of ADSCs.

Session No.: P4-131

Building in Vitro Human Liver Tissue Model for Drug Testing

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In drug industry, huge amounts of chemicals enter the drug screening process each year, but typically Only one drug gets launched into the marketplace ~12 to 15 years later, costing ~$3 to $5 billion. Among the many drug safety issues, hepatotoxicity is often the main concern. At present, although there have been several in vitro hepatocyte models, such as sandwich culture, spheroid culture, micropattern culture, how to maintain hepatocyte functions in long term is still a big challenge. As for animal models, they may not adequately predict the clinical efficacy of therapeutics for certain human tissue types. Therefore, there is a pressing need for the field of tissue engineering to venture into creating realistic three dimensional (3D) human liver tissue models for drug screening purposes. This would help to identify and classify hepatotoxic compounds in the early stages of drug development. Cell sheet technology provide a feasible way to prepare 3D human liver tissue in vitro. Densely distribution of hepatocytes in cell sheet allows close cell-cell contact and sufficient formation of cell junctions, which may in turn result in increase of hepatocyte functions. By stacking hepatocyte sheets and stromal cell sheets, a stratified bioengineered liver tissue could be fabricated, which resembles hepatic cell cord structure in vivo. Our preliminary research have demonstrated that by optimizing seeding density, co-culture ratio and cell types, function of hepatocytes in cell sheet were found to be significantly enhanced. We are now trying to make this cell sheet based liver tissue model become adequate for testing drugs.

Session No.: P4-132

17-B Estradiol Enhances Proliferation and Myogenic Differentiation of Rat Adipose-derived Cells in Vitro

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The increased incidence of stress urinary incontinence (SUI) in postmenopausal women has been proposed to be associated with a reduction in the level of 17-β estradiol (E2). E2 has also been shown to enhance the multi-differentiation ability of adipose-derived stem cells (ASCs) in vitro. However, studies on the potential value of E2 for tissue engineering in SUI treatment are rare. In the present study, we successfully fabricated myogenically differentiated ASCs (MD-ASCs), which were seeded onto a Poly(L-lactide)/Poly(e-caprolactone) electrospinning nano-scaffold, and incorporated E2 into the system, with the aim of improving the proliferation and myogenic differentiation of ASCs. ASCs were collected from the inguinal subcutaneous fat of rats. The proliferation and myogenic differentiation of ASCs, as well as the nano-scaffold biocompatibility of MD-ASCs, with or without E2 supplementation, were investigated. We demonstrated that E2 incorporation enhanced the proliferation of ASCs in vitro, and the most optimal concentration was 10-9 M. E2 also led to modulation of the MD-ASCs phenotype toward a concentrated type with smooth muscle-inductive medium. The expression of early (alpha-smooth muscle actin), mid (calponin), and late-stage (myosin heavy chain) contractile markers in MD-ASCs was enhanced by E2 during the different differentiation stages. Furthermore, the nano-scaffold was biocompatible with MD-ASCs, and cell proliferation was significantly enhanced by E2. Taken together, these results demonstrate that E2 can enhance the proliferation and myogenic differentiation of ASCs and can be used to construct a biocompatible cell/nano-scaffold. These scaffolds with desirable differentiation cells show promising applications for tissue engineering.

Session No.: P4-133

Human Pluripotent Stem Cells Culture on Recombinant Vitronectin-grafted Hydrogel Under Xeno-free Conditions

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Human pluripotent stem cells (hPSCs) are promising cell source for regenerative medicine and therapeutic application. The development of chemically defined biomaterials is necessary for culturing hPSCs for clinical applications without xenogenic contaminants. The feeder-free cultures using synthetic biomaterials offer more reproducible culture conditions and lower the cost of production. We have developed the biomaterials for hPSCs culture to maintain their pluripotency, such as (a) dishes coated with recombinant vitronectin (b) dishes immobilized with oligopeptides derived from extracellular matrices (ECMs). However, the effect of the elasticity of the synthetic dishes on the pluripotency fate and proliferation of hPSCs is still not clear. In this study, we developed polyvinylalcohol-co-itaconic acid (PVA-IA) films grafted with recombinant human vitronectin (rhVN) to evaluate the physical effect of elasticity of hydrogels grafted with biologically active nanosegments on the pluripotency and proliferation fates of hESCs (WAO9). The PVA-IA hydrogels were prepared with different elasticities ranging from 10.3 to 30.4 kPa storage moduli by controlling the crosslinking time with glutaraldehyde. Subsequently, rhVN was grafted on PVA-IA hydrogels with or without carboxymethyl chitosan main chains. We designed to generate the branched type of rhVN chains on PVA-IA hydrogels. This study investigates the optimal elasticity of PVA-IA hydrogels grafted with rhVN that was prepared with much less concentration (5-10 ug/ml) compared to the concentration of oligovitronectin (500-1000 ug/ml) that was used in previous study for the expansion of hPSCs for a long period of hPSC culture (10-20 passages) under xeno-free condition. hPSCs cultured on PVA-IA hydrogels grafted with rhVN were evaluated from pluripotent protein expression by immunostaining, embryoid body (EB) formation, and teratoma formation after 10 and 20 passages.

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Hpsc Culture on Hybrid Hydrogels Under Feeder-free and Xeno-free Culture Conditions

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For recent years, the research in long-term culturing human pluripotent stem cells (hPSCs) including human embryonic stem (ES) cells and induced pluripotent stem (iPS) cells has become crucial due to hPSCs having promising applications in regenerative medicine. Nevertheless, one of the factors causing difficulty of the clinical application of hPSCs is the usage of the expensive culture medium containing growth factors. The reason for this situation can be emphasized on the unstable growth factors in the medium such as FGF-2 and TGF-β1, which are very costly and should be maintained at -70 degree -20 degree. In this study, growth factor-immobilized hybrid hydrogels are developed in order to enable the culture medium to be stored at over zero degree (e.g., 4 degree refrigerator). The design is inspired owing to several articles suggesting that the total amount of growth factor usage can be reduced by immobilization of growth factors on the surface compared to the usage of growth factors in the culture medium. This result is attributed to two reasons: (a) The growth factor-immobilized dishes can be reusable when the culture medium (without growth factors) is exchanged and (b) growth factor immobilized on the dish has higher stability than the growth factor in the culture medium since the growth factor tends to structurally deform in the culture medium . Here we report hPSC culture on heparin-polyvinylalcohol hybrid hydrogels where heparin serves as binding site of growth factors such as FGF-2 and TGF-β1. After immobilization of the growth factors on the heparin-polyvinylalcohol hybrid hydrogels, hPSCs were cultured on the hybrid hydrogels in the culture medium without usage of FGF-2 and TGF-β1 in Essential 6 in xeno-free conditions.

Session No.: P4-135

Pluripotency and Differentiation Ability of Human Adipose-derived Stem Cells Cultured in 2-D and 3-D Culture

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Human adult stem cells, such as human adipose-derived stem cells, are considered to be an attractive source of stem cells than human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). This is because human adult stem cells do not generate the ethical concerns that accompany in hESCs. Although human adipose-derived stem cells (hADSCs) are promising for use in regenerative medicine, their lower expansion ability (aging problem) due to the lower pluripotency of hADSCs compared with hESCs and hiPSCs is a critical issue. We found that the pluripotency gene expression of Oct4, Sox, Nanog, and Klf4 in hADSCs after cultivation on TCPS dramatically decreased compared to those in the cells in stromal vascular fraction (SVF) as well as the cells in 3-D culture. There are high pluripotent stem cells in SVFs and 3-D cultire, although SVF has more heterogeneous population compared to hADSCs cultured on tissue culture polystyrene (TCPS) dishes. There are two theories to explain this phenomenon, one is “Elite model” and the other is “Stochastic model”. Elite model indicates there contains high pluripotent stem cells in SVF and in 3-D culture where high pluripotency stem cells were decreased after cultivation on TCPS. Stochastic model explains that the pluripotency of the same stem cells changes depending on their microenvironment where hADSCs in floating conditions (3-D culture) express high pluripotency gene expression. We evaluated whether hADSCs can be explained by “Stochastic model” or “Elite model” by hADSCs culture in 2-D culture and 3-D culture sequentially. Precisely, we evaluated the difference of gene expression of the cells when hADSCs were cultured in the 2-D condition (TCPS) and in 3-D condition (Ultra low dish) sequentially. We also evaluated the aged hADSCs whether the cells would start the expansion and recover their stemness, after 8 passages when hADSCs expansion was

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hPSC Differentiation into Cardiomyocytes Cultured on Biomaterials Immobilized Nanosegments (ECMs and Oligopeptides)

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Human pluripotent stem cells (hPSC) of human embryonic stem cells (hESCs) and Induced pluripotent stem cells (hiPSCs) have the potential ability to differentiate into many kind of cell types originated from three germ layers : endoderm, mesoderm and ectoderm cells such as dopamine-secreting for Alzheimer disease treatment and insulin-secreting cells for diabetes treatment. However, it is a challenging issue to guide hPSCs to be differentiated into our desired lineages of cells due to their variety of differentiation ability. The fate of differentiation of stem cells is determined by different factors existed in the microenvironment of hPSCs : bioactive molecules, cell-cell interactions, physical factors and cell-biomaterial interaction. It is a reasonable strategy to mimic the stem cell microenvironment for the differentiation of hPSCs into specific lineages of cells using optimal biomaterials for hPSC culture. We selected cardiomyocytes differentiation of hPSCs because it is a relatively easy method to differentiate hPSCs using xeno-free differentiation method. Currently, it has not yet investigated which extracellular matrices (ECMs) or nanosegments derived from ECMs promote hPSCs differentiation into cardiomyocytes. We developed nanosegment-grafted biomaterials having different elasticity for hPSCs differentiation into cardiomyocytes. In this study, we prepared ECM-coated dishes, PVA-IA(polyvinylalcohol-co-itaconic acid) hydrogel dishes having different elasticity that are grafted with several ECMs with different surface density, and PVA-IA hydrogel dishes having different elasticity that are grafted with several oligopeptides with different surface density. The chemically defined protocols for cardiomyocyte induction from hPSCs will be used in clinical application and in the investigation of molecular mechanism of specification and maturation of cardiomyocytes. We evaluated the optimal elasticity of biomaterials and preferable nanosegments immobilized on the biomaterials for differentiation of hPSCs into cardiomyocytes.

Session No.: P4-137

Enhanced Neuronal Differentiation of Human Induced Pluripotent Stem Cells in Extracellular Matrix Microenvironment

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Human induced pluripotent stem cells (hiPSCs) reprogrammed from human somatic cells by delivering several defined factors provide not only therapeutic cell sources for neuronal regeneration, but biomedical platforms for drug screening and in vitro disease modeling of neuronal disorders. Although diverse methodologies have been applied to enhance neuronal differentiation of hiPSCs, low differentiation efficiency still remains a challenge. To overcome such limitations, here we report a microenvironmetal control using extracellular matrix (ECM) for promoting neuronal differentiation of hiPSCs in vitro. Two different formats of 2D ECM-coated surface and 3D ECM hydrogel significantly facilitated neuronal lineage differentiation of hiPSCs as confirmed by upregulated expression of several neuronal markers and highly matured neuronal morphologies. This study demonstrates that reconstituted ECM microenvironment can provide an efficient platform to produce functional neuronal lineage cells from hiPSCs. This work was supported by a grant from the Korea Health Technology R&D Project funded by the Ministry of Health and Welfare (HI14C1588), Republic of Korea and Brain Korea 21 plus (BK21PLUS) program. Ann-Na Cho, Jung Seung Lee and Jin Kim are fellowship awardee by BK21PLUS program.
Keywords: Human induced pluripotent stem cells, Neuronal differentiation, Extracellular matrix

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Micro or Nano Patterning Control Human Adipose Derived Stem Cell (HASC) Differentiation

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Biophysical cues (e.g., patterns, flexibility and porosity of the cell culture substrates) have been seen as a potential approach to control the modulation of cell behavior. Topography on the substrate like grooves can guide stem cells fate and lead to various cell types. This study devoted to investigating the potential of topography on the differentiation of human adipose-derived stem cells (hASCs) into chondrogenic, osteogenic, adipogenic and neural lineages. Different patterns were fabricated on the silicon wafer by lithography, and stamped on the soft substrates such as gelatin. Then, hASCs were cultured and differentiated on the patterned substrates treated with growth factors to increase cell adhesion.

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Understanding the Regulatory Mechanism of Compression-Induced Mechanopodia in Human Mesenchymal Stem Cells

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Mechanical signal is an important factor that can affect cell fate but the mechanism on how cells respond and adapt to mechanical loading is not well understood. Our previous study has reported the formation of mechanoresponsive, omni-directional and local matrix-degrading actin protrusions, termed mechanopodia, in human mesenchymal stem cells (hMSCs) microencapsulated in collagen upon cyclic compression in order to understand their unique identity and the signaling mechanism behind the formation of these protrusions. While it is still unknown whether they are different from other major types of actin protrusions (such as filopodia, podosomes, invadopodia) on the cell surface membrane, mechanopodia differ in terms of the stability, abundance and major molecular markers. Understanding the signaling mechanism regulating the formation of these mechanopodia is essential. It's been known that small GTPases of the Rho subfamily such as Cdc42 and Rac1 regulate the formation of actin filaments. This study aims to reveal the dependence of mechanopodia formation on Cdc42 and Rac1 signaling. The inhibition of Cdc42 by incubating the hMSCs in ML141 did not prevent the formation of the mechanopodia, while Rac1 inhibition with NSC23766 resulted in a drastic change in morphology with the apparent absence of mechanopodia. This suggests that mechanopodia formation may indeed be dependent on the presence of Rac1 but not on Cdc42. More in-depth analyses are needed to confirm the signaling regulation of mechanopodia and to reveal their functional and physiological significance.

Session No.: P4-140

Enhancement of Cell Adhesion, Retention, and Survival of HUVEC/cbMSC Aggregates That Are Transplanted in Ischemic Tissues by Concurrent Delivery of an Antioxidant for Therapeutic Angiogenesis

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A recurring obstacle in cell-base strategies for treating ischemic diseases is the significant loss of viable cells that is caused by the elevated levels of regional reactive oxygen species (ROS), which ultimately limits therapeutic capacity. In this study, aggregates of human umbilical vein endothelial cells (HUVECs) and cord-blood mesenchymal stem cells (cbMSCs), which are capable of inducing therapeutic angiogenesis, are prepared. We hypothesize that the concurrent delivery of an antioxidant N-acetylcysteine (NAC) may significantly increase cell retention following the transplantation of HUVEC/cbMSC aggregates in a mouse model with hindlimb ischemia. Our in vitro results demonstrate that the antioxidant NAC can restore ROS-impaired cell adhesion and recover the reduced angiogenic potential of HUVEC/cbMSC aggregates under oxidative stress. In the animal study, we found that by scavenging the ROS generated in ischemic tissues, NAC is likely to be able to establish a receptive cell environment in the early stage of cell transplantation, promoting the adhesion, retention, and survival of cells of engrafted aggregates. Therapeutic angiogenesis is therefore enhanced and blood flow recovery and limb salvage are ultimately achieved. The combinatory strategy that uses an antioxidant and HUVEC/cbMSC aggregates may provide a new means of boosting the therapeutic efficacy of cell aggregates for the treatment of ischemic diseases.

Session No.: P4-141

Chitosan-formed-amniotic Fluid-derived Stem Cell Spheroids Exhibit High Immunomodulatory Activity

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Mesenchymal stem cells (MSCs) are a type of adult stem cells which can differentiate into mesoderm layer and exhibit immunosuppressive character. Our previous study demonstrated that amniotic fluid-derived stem cells (AFSCs) isolated from pregnant C57BL/6 mouse exhibit in vitro immunosuppressive effects on allogenic immune cells, but not enough to reduce severity of graft-versus-host disease (GvHD) after allogenic bone marrow transplantation (BMTx). Hence, the purpose of study is to find the optimum condition for culturing AFSCs in vitro that can increase their immunomodulatory effects. Application of three-dimensional (3D) cell culture techniques in stem cell research has been shown to create an “in vivo-like” microenvironment which better preserves MSCs phenotype and innate properties. Therefore, in present study, we examine whether AFSCs could also aggregate to form spheroids on different biomaterial sources and analyze their anti-inflammatory effects of AFSCs spheroids on murine macrophage cell line, RAW 264.7. Results have shown that AFSCs spheroids from chitosan film exhibited better differentiation capability than AFSCs from adherent monolayer cultures or hanging drop (HD) cultures. Conditioned medium (CM) from chitosan film 3D cultured-AFSCs showed better anti-inflammatory effects than that of 2D-cultured AFSCs and HD 3D-cultured-AFSCs on LPS+IFN-γ-stimulated RAW 264.7 by inhibiting secretion of pro-inflammatory cytokines, lipid mediators, chemokines, nitric oxide production and ROS production. In addition, 3D-chitosan-CM increased the secretion of anti-inflammatory cytokines IL-10 and activated M2 anti-inflammatory-macrophage factor, Arginase-1 in macrophages after stimulation. Mechanical studies showed that the high level of anti-inflammatory protein TSG-6, STC-1, and enzyme COX-2 were detected in 3D-chitosan-cultured-AFSCs spheroids. These results suggested that AFSCs cultured as 3D spheroids on chitosan films can increase their anti-inflammatory properties on macrophages.

Session No.: P4-142

Direct Conversion of Endothelial Cells into Osteogenic Cells via BMP-4 Dependent EMT Pathway

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Transdifferentiation is the direct conversion from one somatic cell type into another desired somatic cell type. This reprogramming method offers an attractive approach for regenerative medicine. Here, we demonstrate that human umbilical vein endothelial cells (HUVECs) can be transdifferentiated into osteogenic cells via non-viral delivery of minicircle DNA containing Oct4, Sox2, Nanog, and LIN28 polycistronic genes followed by the bone morphogenetic protein-4 (BMP-4) treatment. Fluorescence-activated cell sorting analysis revealed transfected cells with minicircle DNA. These transfected cells were then allowed to expand for 6 or 48 hours with or without BMP-4, and we observed a significant endothelial to mesenchymal transition (EMT) transcription markers such as a-SMA via BMP-4 treatment. Furthermore, when the BMP-4 treated cells were exposed to osteogenic medium for additional 2 weeks, alkaline phosphotase showed differentiated cells. These results suggested that the induced osteogenic cells were functional in vitro. Taken together, we successfully demonstrated the direct conversion of HUVEC into osteogenic cells by transduction of reprogramming factors followed by BMP-4. The directed differentiation of endothelial cells into osteogenic cells may have significant utility in vascularized bone tissue engineering applications.
KEYWORDS: Transdifferentiation, HUVEC, Osteogenic differentiation, BMP-4

Session No.: P4-143

Macrophages Induced Osteogenesis Under Infection

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Infection is generally considered to be one of the major causes of bone loss; however, under certain conditions, abnormally induced bone formation could be observed in infection-induced inflammatory diseases, such as osteomyelitis and periapical lesions. The mechanism underlying this phenomenon is largely unknown. Previous studies have proved the essential role of immunoregulation in osteogenesis. Macrophage, the key player in the innate immune response against infection, has been found to interact with the osteoblast-lineage cells (also known as bone marrow stromal cells, BMSCs) to regulate bone formation. This study aimed to investigate the interaction between macrophages and BMSCs under infectious conditions. Our in vivo results showed that abnormal calcium deposition was formed in human periapical lesions, which was accompanied with the macrophage infiltration. The in vitro results showed that the osteogenic markers of BMSCs were significantly up-regulated when the BMSCs were co-cultured with macrophages with the supplementation of LPS (to simulate infection); the ALP activities and the formation of mineralization nodules were induced accordingly. This study demonstrated that in infection-induced inflammatory diseases, macrophages could be activated and interacted with BMSCs to induce the osteogenic process. Our study partially explained the mechanisms of the abnormal bone formation in infection-related diseases.

Keywords: Macrophage, Bone formation, Infection

Session No.: P4-144

Blood Vessel Three-dimensional Reconstruction of a Tissue Engineering Nerve Bridging Rat Sciatic Nerve Injury

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To do the reconstruction of blood vessel three-dimensional image of tissue engineering nerve bridging rat sciatic nerve injury and relative parameter analysis by a method of MICROFIL perfusion and Micro-CT scanning. Tissue engineering nerves were constructed in vitro with Schwann cells differentiated from skin-derived precursors (SKPs) (SKP-SCs) of rats as supporting cells and chitosan nerve conduits combined with silk fibroin fibers as scaffolds to bridge 10mm sciatic nerve injuries in rats compared with autologous nerves. At 4 weeks after surgery, blood vessel three-dimensional reconstructions following MICROFIL perfusion and Micro-CT scanning, and parameter analysis of tissue engineering nerves and autologous nerves were conducted. In the blood vessel three-dimensional images of tissue engineering nerves and autologous nerves, a large number of microvessels and capillaries were displayed well relatively. Through the parameter analysis of blood vessel three-dimensional images, a few of parameters were obtained, which reflected the number, diameter, connection and spatial distribution of blood vessels. The average diameter of neovasculars in tissue engineering nerves was less than that in autologous nerves, the difference of which was statistically significant. However, the other parameters had no significant differences. Neovasculars were mainly capillaries and microvessels, the diameters of which range from 9μm to 301μm in tissue engineering nerves and range from 9μm to 228μm in autologous nerves. Microvessels of tissue engineering nerves implanted in vivo could be displayed well relatively by the method of MICROFIL perfusion and Micro-CT scanning, which is a feasible means to evaluate and compare the differences and changes of angiogenesis of tissue engineering nerves implanted in vivo under different conditions.

Session No.: P5-145

Injectable Thermosensitive Chitosan-based Hydrogels for Temporomandibular Joint (TMJ) Tissue Engineering and Regeneration

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Temporomandibular disorders (TMD) are a group of degenerative joint disease and a major cause of orofacial pain. There is a high prevalence of TMD in the general population with an estimated 40–75 % having at least one sign and 33 % reporting at least one symptom of TMD. Current treatment options are limited and do not present structure-modifying benefits. The emerging field of TMJ tissue engineering aims to facilitate intra-articular delivery of cells, growth factors and/or drugs to promote TMJ regeneration. In this study, injectable thermosensitive hydrogels based on chitosan (C), gelatin (G) and beta-glycerophosphate (BGP) were designed. The gel formation, rheological properties, morphology, pH, degradation, cytotoxicity and protein release characteristics of this gel system were investigated. Five gel formulations of varying chitosan-to-gelatin mass ratios at 0.5% (w/v) BGP were fabricated. Notably, rheological analysis showed a reduced gelation time and a higher storage modulus (2.9±0.1 sec; 656.0±38.7 Pa) in the hydrogels made with 2:1 chitosan-to-gelatin mass ratio compared to the chitosan-only gels (76.7±10.1 sec; 187.7±37.0 Pa). Scanning electron microscopic analysis further revealed higher densities and reduced pore sizes with increasing amounts of gelatin, as a result of hydrogen bonding and electrostatic interaction between chitosan and gelatin. Furthermore, with the addition of gelatin, TMJ disc cells seeded in C-G-BGP hydrogels demonstrated enhanced cell viability and proliferation compared to that in the chitosan-only gels. As BGP is cytotoxic at high concentrations, incorporation of gelatin provides a more viable approach to improve the properties of the hydrogel. In summary, the natural C-G-BGP hydrogels are promising injectable matrices that may be useful for TMJ drug delivery and tissue engineering.

Session No.: P5-146

The Osteoblast-like Cell Activity on Ca-P Modified Zirconia by Liquid Precursor Infiltration Technique

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Introduction: Zirconia ceramics have been introduced into dentistry as a metal replacement for implant, but its bioinertness causes many problems. It is necessary to improve its biological activity.

Materials and Methods: The pre-sintered zirconia discs(13 mm in diameter and 1.5 mm in thickness) were randomly divided into 4 groups: Z0 as control group, Z1, Z2 and Z3 were immersed in the Ca-P precursor solution for 1, 2 and 3 minutes, respectively. After dense sintering, another 4 subgroups was divided from above 4 groups named as Z0h, Z1h, Z2h and Z3h, which were hydrothermal treated. Morphology and composition of these groups were investigated by Scanning Electron Microscopy and Energy Dispersive Spectrometer; Cross-section morphology of group Z2 was evaluated with SEM. In vitro osteoblast-like cell (MC3T3-E1) was used to evaluate the cell proliferation kinetic and ALP activity of the groups; Statistical analysis was performed using an ANOVA test, the results were taken to be significant at a probability level of $P<0.05$.

Results: Experimental groups showed Ca-P clusters on their surfaces, and the distribution densities of clusters were increased with the extension of immersion time. Ca-P clusters turned into rod crystals after hydrothermal treatment. The EDS data showed a molar ratio about 1.67 at the area of rod crystals in hydrothermal treated groups; Ca-P clusters infiltrate into zirconia with a depth of approximately 8 um and crossed each other. The cell morphology changing to flat was observed on group Z2h at 24h cultivation, the cell proliferation on group Z2h was significantly higher than group Z2 at 7 days, the cell ALP activity of Z2h was high significantly compared with that of Z0 and Z2 at 7, 14 days ($p<0.05$).

Conclusions: This studies offers an effective way to modify the zirconia surface to improve its biocompatibility.

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Session No.: P5-147

Synthesis and Characterization of Stimuli-responsive Biodegradable Polypeptide Hydrogel

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A novel pH- and thermo-responsive block copolymer based on polypeptide was synthesized by ring-opening polymerization of γ-benzyl-L-glutamate-N-carboxyanhydride (BLG-NCA) with PEG-diamine as a macroinitiator and followed by aminolysis reaction. The resulting biodegradable and biocompatible tri-block copolymer, poly[2-(dibutylamino)ethyl-L-glutamate]-poly(ethylene glycol)-block-poly[2-(dibutylamino)ethyl-L-glutamate] (PNLG-b-PEG-b-PNLG), showed a low viscosity sol state at low pH (pH-6.4) or at low temperature (250C) but it formed good gel at physiological condition (pH-7.4 and 370C). The gelation behaviors could be control by changing the ratio between hydrophobic moiety and pH-sensitive moiety. The structure of the PNLG-b-PEG-b-PNLG copolymer was confirmed by H-NMR and gel permeation chromatography (GPC). However the hydrogel solution (30wt% and pH-6.4) formed strong gel instantly without major inflammation when it injected subcutaneously into the Sprague–Dawley (SD) rats. The PNLG-b-PEG-b-PNLG copolymer did not show any cytotoxicity in vitro. So PNLG-b-PEG-b-PNLG is a potential candidate for drug and gene delivery. Keyword: Polypeptide, biodegradable, pH- and thermo-responsive hydrogel. References: 1. C. Deng et al., Prog. Polym. Sci., 2014, 39, 330–364. 2. Yi Li et al., J. Polym. Sci. A: Polym Chem., 2013, 51, 4175–4182. 3. Anika M. Jonker et al., Chem. Mater., 2012, 24, 759−773. 4. Shusheng Zhang et al., Polym. Chem., 2014, 5, 3346–3351.

Session No.: P5-148

Synthesis and Characterization of Injectable in Situ Enzymatically Serum Hydrogel

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A phenolic hydroxyl group was incorporated into albumin, using aqueous carbodiimide activation chemistry, to obtain in situ gellable and injectable albumin-based materials for customized tissue engineering applications. By this means, albumin derivatives that were gellable via a peroxidase-catalyzed reaction were obtained. The enzymatically cross-linked albumin gels showed tunable storage modulus and proteolytic degradability. The time necessary for gelation decreased with increasing content of the phenolic hydroxyl (Ph) group, peroxidase concentration and decreasing H2O2 concentration. We further synthesized autologous serum hydrogels that retained its integrity in vivo for more than one month and was eventually degraded due to hydrolysis. The synthesized plasma hydrogels exhibited little cytotoxicity and hemolysis; the acute inflammatory response after implanting the hydrogel was negligible, and the degradation products were biocompatible. A mouse model of sidewall defect-bowel abrasion was employed, and a significant reduction of post-operative peritoneal adhesion has been found in the group of in situ formed plasma hydrogels.

Session No.: P5-149

Bioinspired Zwitterionic Surface Coatings with Robust Photostability and Fouling Resistance

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Zwitterionic materials have been widely implemented in surface modification and as biomaterials that exhibit exceptional hydrophilicity, biocompatibility, and antifouling properties. Here we report a new superhydrophilic adsorbate, L-cysteine betaine (Cys-b), which was obtained by quaternization of amino group of L-cysteine. Cys-b exhibits enhanced chemical stability, biofouling resistance, and inertness to environmental changes. Gold substrates modified with Cys-b exhibit excellent suppression of photoinduced oxidation, resistance to nonspecific proteins absorption, bacteria, and fibroblast cells. In addition, plasmonic hollow Ag@Au nanoshells modified with Cys-b show good colloid stability in the presence of Cu2+, and can be readily used for hyperthermia applications. Overall, these properties of Cys-b make it become a promising candidate for medicine device's coating material.

Session No.: P5-150

Functionalization of Polydopamine via the Aza-Micheal Reaction for Antimicrobial Interfaces

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Polydopamine (pDA) coatings afford tremendous versatility because of their capabilities to provide substrate-independence functionalization with a wide range of amine- and thiol-containing molecules. In this work, a new and facile conjugation approach to the formation of β-amino carbonyl linkages between pDA and acrylate/ acrylamide molecules via the aza-Micheal reaction. Sulfobetaine acrylamide (SBAA), sulfobetaine methacrylate (SBMA) and poly(ethylene glycol) methacrylate (PEGMA) were used to graft onto pDA films to enhance the antifouling function. The coating strategy was applied to different substrates such as TiO2, SiO¬¬2, Nitinol alloy, polystyrene and poly(dimethyl siloxane). X-ray photoelectron spectroscopy (XPS) and water contact angle measurements were applied to observe the variation of surface chemistry and surface wettability upon pDA modification and subsequent conjugation. Antifouling properties of coating were challenged by three gram-positive and gram-negative bacteria. Cytotoxicity of coatings on NIH-3T3 fibroblast cells were assessed by MTT assay. The results showed that the SBAA-grafted surfaces is not only effectively reduce the adherent bacteria but also reduce the contact angle. In addition, multifunctional pDA by integrating antifouling properties into a coating, using Ag nanoparticles followed by the grafting on SBAA for bacterial repellence. The data showed that the persistent release occurred over the experiment time span, which imply the feasibility of a long-term use of antimicrobial coatings. Consequently, this work open a new horizon to the grafting strategy to engineer pDA and the functional bioinspired antifouling interfaces in a substrate-independent fashion.

Session No.: P5-151

A Versatile Approach to Antifouling and Substrate-independent Coatings via Assembly of Metal-phenolic Networks

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The design of antifouling and biocompatible surfaces to resist nonspecific attachment of proteins, cells and bacteria is crucial for applicability of medical devices, such as biosensing and medical implant. In this study, we report a facile and versatile approach to fabricate an antifouling coating via coordination of polyphenols and Fe(III) ions in an aqueous solution to address common fouling problems on a variety of substrates.1 This approach incorporates bioinspired zwitterionic sulfobetaine dopamine (SB-DA) for fouling resistance, tannic acid (TA) and Fe(III) ions, serving as crosslinking agents. Film formation is triggered by the adsorption of the TA-Fe(III) complex network on various planar surfaces including organic and inorganic substrates. Because of super hydrophilic and charge-balanced properties, SB-DA enables forming a tightly bound water layer on the top of the complex network to repel nonspecific adsorption.2 The surface hydration and the chemical states of the modified substrates were confirmed by contact angle goniometer and X-ray photoelectron spectroscopy. For examining the antifouling properties, we immersed the modified substrates into the solutions containing bovine serum albumin or bacteria, and then the adsorbed proteins and bacteria were quantified using ELISA and cell imaging analysis. Consequently, this approach for substrate modification offers an easy, fast and environment friendly way to realize substrate-independent biocompatible coatings for all types of materials. The work also provides insight into the construction of hierarchical structures by molecular assembly for functional biointerfaces.

Session No.: P5-152

Bio-Inspired Multifunctional Catecholic Assembly for Photo-programmable Biointerface

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This project reports a novel multifunctional mussel-inspired zwitterionic catecholic assembly to form a photoresponsive biointerface. The assembly is the combination of the antifouling sulfobetaine and photocleavable o-nitrophenyl moieties into a molecule, becoming sulfobetaine nitrodopamine (SB-nDA). We demonstrated the formation of a compact thin SB-nDA film on TiO2 by using the pH transition approach. The film thickness, surface wettability and elemental composition were characterized using ellipsometry, contact angle goniometer, atomic force microscopy and X-ray photoelectron spectroscopy, respectively. The SB-nDA thin films can effectively resist adhesion of both Gram-positive Staphylococcus epidermidis and Gram-negative Pseudomonas aeruginosa by more than 95% relative to bare TiO2. Quartz crystal microbalance with dissipation (QCM-D) sensor was employed for protein fouling tests, showing the comparable antifouling property of SB-nDA with thiol- or silane-based surface ligands. More importantly, the spatiotemporal control over the bioinertness by UV irradiation has been studied with bacterial and protein adsorption. Therefore, the catecholic chemistry can be used for programmable tailoring of interfacial properties, permitting potential application in light-guided targeting for nanomedicine.

Session No.: P5-153

Surface Modification of Silicon Elastomer with Zwitterionic Silane for Improved Antifouling Applications

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Biofouling is a serious problem in many applications ranging from biosensors to biomedical implants and devices, and from food packaging to industrial and marine equipment. To address these problems, we developed a stable superhydrophilic zwitterionic interface on polydimethylsiloxane (PDMS) elastomer as a self-assembled layer (SAL). Static water contact angle, and photoelectron spectroscopy were used to investigate the wettability and Surface elemental composition of the modified silicon surfaces. The substrates were exposed to bacteria, protein, and lipid solutions to study the antifouling properties. The results showed an excellent biofouling-resistance properties for the modified substrates even after 30 days storage in ambient environment. Furthermore, the cytotoxicity of SBSi was studied using NIH-3T3 fibroblast by the MTT assay, showing a negligible cytotoxicity effect. On the other hand, commercially available silicon hydrogel contact lenses were modified with the developed zwitterionic silane. The properties of these anti-biofouling contact lenses were investigated, which indicated that the modified contact lenses had superior antibacterial adhesion properties. Additionally, the improvement in the biocompatibility and stability of this surface modification on silicone-based medical devices with SBSi allows a wide range of applications, particularly implants for in vivo uses.

Session No.: P5-154

Promotion of Salivary Organoid Generation from Human Glandular Stem Cells Using Biomimetic Three-dimensional Spheroid Culture

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INTRODUCTION: Development of a tissue-engineered, salivary bio-gland will benefit patients suffering from xerostomia due to loss of fluid-secreting acinar cells. This study was conducted to develop a bioengineering system to generate glandular organoids from human salivary glandular stem cells (hSGSCs) through three-dimensional (3D) spheroid culture. METHODS & RESULTS: Microwells were fabricated by photopatterning of PEG hydrogel in the presence of an electrospun PCL nanofibrous scaffold. hSGSCs were plated on plastic dishes, PCL nanofibers, or PCL nanofibrous microwells. Upon a differentiation induction, hSGSCs cultured on PCL nanofiber and PCL microwells aggregated to form 3D acinar-like organoids and the greatest differentiation potency was observed on the PCL microwells. The 3D-assembled organoids in the PCL microwells expressed higher levels of salivary epithelial markers (α-amylase and AQP5) and tight junction proteins (ZO-1 and occludin) than those on the PCL. Furthermore, the 3D-assembled organoids in the PCL microwells showed higher levels of transepithelial electrical resistance (TER), α-amylase secretion and intracellular calcium concentration ([Ca2+]i) than those on the PCL nanofibers, suggesting more robust and functional organization to polarized salivary acinar units from hGSCs. DISCUSSION & CONCLUSIONS: We established a bioengineering 3D spheroid culture system to generate robust and functional acinar-like organoids from hSGSCs. PCL nanofibrous microwells can be applied in the future for bioengineering of an artificial bio-salivary gland for restoration of SG function.

Session No.: P5-155

Effect of Centrifugation on the Collagen and Gags within the Decellularized Adipose Tissue

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Decellularized Adipose Tissue (DAT) was a potential regenerative material for soft tissue engineering. However, the outcome of regenerative was effect by collagen and glycosaminoglycan (GAGs) content within the DAT. In this study, we purposed to improve the procedure of DAT with less cell content and more percentage of glycosaminoglycan and collagen by centrifugation. After different processing, the fat tissue was quantitative analyzed for nucleic acid, GAGs and collagen. It was concluded that compared with SDS . After centrifuging, the percentage of GAGs in matrix was largest increased in 600 rpm, and the largest percentage of Collagen was in 900 rpm. According to the results, we speculate that centrifuging in 600 to 900 rpm can have a good effect on purifieding the fat.

Session No.: P5-156

Development of Optical Cell Collection System Combining Photodegradable Hydrogel Cell Image Analysis

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Recently, in production of therapeutic cells for regenerative medicine, and in construction of cell assay system for drug evaluation, it is important to understand heterogeneity in a cell population. Conventionally, a fluorescent label is an effective tool to distinguish target cells from the other cells. However, since labeling technology is commonly based on biological markers, it is difficult to profile cells which do not have clear cellular marker, or which are not certain for their maturation status. Primary cells from patient tissue tend to show such heterogenic and complex cellular population. They can consist of mesenchymal cells in different status, endothelial cells, and stem cell or cancerous cells that were hidden in niches. Therefore, categorization of different cell types has been an challenge. We consider that to detect and collect the characteristic sub-populations in heterognic cells, label-free morphology-based analysis method is a powerful tool. In this work, to understand the heterogeneity of cells, we developed automated cell collection system, which combines “3D hydrogel culture condition”, “image analysis”, and “robotic pipetting system”. For hydrogel, we have developed new photodegradable gelatin hydrogels by crosslinking gelatin through the reaction between ‘amino groups’ in gelatin and ‘N-hydroxysuccinimide (NHS)-terminated photocleavable o-nitrobenzyl groups for optical separation of single-cell-derived cell aggregate which shows characteristic morphology (Sci. Rep., 5, 15060 (2015)). For image analysis, we have applied our clustering-based morphology evaluation using multiple morphological parameters. By evaluating 3D cultured cells, we demonstrate that our system can classify single-cell derived 3D cell aggregates with their real-time proliferation profiles, and clone them as morphologically categorized libraries for further characterization.

Session No.: P5-157

Pellet Culture for Chondrogensis Using Concave Microwell Plate

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Pellet or micromass culture is an in vitro technique that has been used widely to study in vitro chondrogenesis. While pellet cultures are usually established in a polypropylene centrifuge tubes, it can be more cumbersome, time consuming and takes a great amount of culture media when multiple samples. We have recently developed a culture plate that has 25 concave microwells to substitute culture tube. In this study, we compared the in vitro chondrogenesis from bone marrow-derived mesenchymal stem cells using concave microwell plates with that obtained using culture tubes. Pellets cultured in concave microwell plate were larger than those cultured in tube, significantly on day 21. Pellets cultured in concave microwell plate had significantly higher level of GAG per DNA content and greater proteoglycan content that those cultured in tube at days 7 and 14. Three chondrogenic markers showed significantly higher expression from pellets cultured in concave microwell plate than those cultured in tubes at day 7 and 14. At day 21, there was not a significant difference in the expression of threes markers. COL10A1, the typical hypertrophy marker, was significantly lower in concave microwell plate during the whole culture period. Runx-2, a marker of both hypertrophy and osteogenesis, was significantly lower at day 7 in pellets cultured in concave microwell plate than those cultured in tube. In conclusion, early and enhanced chondrogenesis was obtained with the use of PDMS concave microwell plate compared with the use of culture tube. This concave microwell plate provides a convenient and effective tool for the study of in vitro chondrogenesis and may replace the use of propylene culture tube. ACKNOWLEDGEMENTS: This study was supported by the National Research Foundation (NRF) funded by the Korean government (2015R1A2A1A09002793 and 2013R1A1A2062961).

Session No.: P5-158

Plasma Immersion Ion Implantation Treatment of Peek for the Immobilization of Biomolecules

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Introduction: Poly-ether ether ketone (PEEK) has emerged as a leading biomaterial due to its biocompatibility and mechanical properties. However, due to the inertness of PEEK, bioactivity remains suboptimal and interest had been focused on improving this aspect. Plasma Immersion Ion Implantation (PIII) was demonstrated to improve the bioactivity of many polymers while providing a one-step method to covalently immobilize proteins onto the treated surfaces. The aim of this project is to apply PIII treatment on PEEK and explore methods of achieving better cell responses. Experimental: The modified surfaces were evaluated by means of ELISA, gBCA and ATR-FTIR detection coupled with SDS washing. Cell culture studies including attachment, proliferation, gene expression and bone mineralization were performed to assess the biological responses. Results: Protein studies demonstrated that proteins remain detectable on the surfaces after harsh washing conditions confirming that proteins are covalently linked to the modified surfaces. Cell culture results show that the treated surfaces alone increased the bone cell attachment rate by over 80% with significantly improved spreading and proliferation. The covalently linked ECM proteins on the treated surfaces were also shown to remain highly biologically active. Conclusion: The current results had shown that cell attachment to PEEK can be largely improved by using PIII. These findings will help us further understand the effects of the PIII treatment process on PEEK in which the outcomes may help us improve its bioactivity for bone related applications.

Session No.: P5-159

Ph-responsive 3D Scaffold for Stem Cell Culture

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A new scaffold that is responsive to pH has been developed by a new technology. Different from the previous technology, we lay salt particles at the top and bottom of the polymer-salt particle paste and make a sandwich tablet, after salt leaching, we can get a scaffold with porous surface. The scaffold can maintain its porous structure in pH 7.4 cell culture, after several days' proliferation, dissolve the scaffold in pH=6.0 PBS solution. Then by centrifuge and filter, we can get the cells. The polymer has been proved to have a good solubility at pH 6.0. And the scaffold have been characterized by SEM. This scaffold overcomes the problem to synchronize the rate of scaffold degradation and cell proliferation and find a new way for easy separation scaffold.

Session No.: P5-160

Preparation of Poly(Vinyl Alcohol) and Carboxymethyl Cellulose Biocomposites

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A biomimetic composites made of poly(vinyl alcohol) and carboxymethyl cellulose (PVA/CMC) hydrogels were proposed. The PVA/CMC hydrogels were prepared by freeze-thawed cycles that the phase separation of polymer solutions during freezing stages led to the formation of crystallites and resulted in the insoluble hydrogels. The PVA/CMC hydrogels contain at least 71% of insoluble gels found in gel fraction. Moreover, the gel fraction and swelling ratios were clearly altered by the composition of PVA and CMC. Four groups of samples were prepared: pure PVA, P2C1 (containing two thirds of PVA and one third of CMC), P1C1, and P1C2 hydrogels. For pure PVA hydrogels, the swelling ratio was 416 %, while the P1C2 hydrogels exhibited a much higher swelling ratio (1437 %). In summary, an insoluble hydrogel composed of PVA/CMC with excellent physical and mechanical properties. The prepared hydrogels showed the potential applications for tissue engineering scaffolds.

Session No.: P5-161

Bacterial Cellulose-collagen Composite Scaffolds for Bone Tissue Engineering

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Tissue-engineered scaffolds take a very important position in that they can provide a 3D cellular environment. Collagen (Col) scaffold has been a good candidate and have found many applications in tissue engineering. However, weak mechanical property of Col itself limits more extended applications in tissue regeneration. Here we propose a more robust system using Col and bacterial cellulose (BC). In this study, BC/Col composite scaffold was fabricated and their effect was investigated using umbilical cord blood derived mesenchymal stem cells (UCB-MSCs), along with collagen scaffold as a control. The BC pellicles were crushed to form a BC slurry by using a homogenizer. Then, the BC slurry was simply mixed with the 1% collagen. The slurry mixture (BC/Col) solution were then freeze-dried overnight. The BC/Col scaffolds were characterized via scanning electron microscopy and Fourier transform-infrared spectroscopy. They were further analyzed for the measurement of mechanical property. After UCB-MSCs were seeded into the BC/Col scaffolds, they were subjected to osteogenic differentiation in vitro. We found that cells were viable and proliferative in the BC/Col scaffold. In addition, when osteogenic differentiation of UCB-MSCs was examined for up to 21 days, alkaline phosphatase (ALP) activity and osteogenic maker (COL1, ALP, osteocalcin, bone sialoprotein) expression were significantly improved with UCB-MSCs cultured in the BC/Col scaffold. The proposed BC/Col composite scaffold should be considered another option for bone tissue engineering.

Session No.: P5-162

Cell-derived Extracellular Matrix-coated Polymer Mesh Scaffold for Bone Tissue Engineering

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Tissue-engineered scaffolds are required to mimic the natural structure and biological function of the extracellular matrix (ECM) and to retain mechanically supportive property. However this goal is still a very challenging task. In this study, porous PLGA/PLA mesh scaffold is prepared and then treated using cell-derived ECM (CDM). CDM is known to have compositional characteristics very similar to a complexity of natural ECM. These CDM-coated mesh scaffolds are assessed for their potential as an osteogenic 3D microenvironment for human umbilical cord blood-derived mesenchymal stem cells (UCB-MSCs). CDM was obtained via decellularization in which in vitro-cultured type I collagen overexpressing (Col I -293T-DK) cells were treated with mild detergents and enzymes (DNase and RNase). CDM was then homogenized and used for mesh scaffold coating. The presence of CDM was confirmed via scanning electron microscope and fibronectin (FN) immunofluorescence. After then, UCB-MSCs were seeded into the scaffolds and the cell-loaded scaffolds were cultured for 21 days in an osteogenic condition for the induction of osteogenesis of UCB-MSCs in vitro. Experimental groups are mesh scaffold itself (control), FN-coated mesh scaffold (FN-mesh), and CDM-coated mesh scaffold (CDM-mesh). We found out that most of the cells are viable and better proliferative on CDM-mesh scaffold. In addition, when osteogenic differentiation of UCB-MSCs was evaluated, osteogenic markers (COL I, ALP, osteocalcin, bone sialoprotein) expression and alkaline phosphatase (ALP) activity were significantly improved with UCB-MSCs when cultured in the CDM-mesh scaffold compared to the control and FN-mesh. Polymer mesh scaffolds coupled with CDM provide UCB-MSCs with a suitable microenvironment for osteogenesis in vitro and indicate the significant but unidentified roles of CDM-MSCs interactions.

Session No.: P5-163

Dielectrophoretically Aligned Microcapsules with Encapsulating Bioactive Molecular to Control Microenvironment of Hydrogel for Electroactive Tissue Engineering

Min-Yu Chiang[1], Yi-Zhen Lin[1], San-Yuan Chen[1]

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Hydrogels are often used as scaffolds in the field of tissue engineering due to their high water content, biocompatibility, and biodegradability. For the electroactive tissue engineering regeneration, i.e. muscle, cardiac and neural tissue, hydrogels might usually require additional properties, such as electrical conductivity, mechanical, and controllable bioactive properties, by incorporating nanomaterials or modifying biochemical molecular. To control the mechanical, electrical, or topographical properties of hydrogels, various strategies are prone to controlled the orientation of nanomaterials in hydrogel, including carbon nanotubes, silicon oxide nanowires, gold nanowires, and magnetic particles to improve the microenvironment of hydrogel. However, these hydrogels with usually lack specific bioactive functions for mimicking native electroactive tissues, such as encapsulation and controllable delivery of biological and chemical cues. In this study, we developed a hydrogel scaffold with dielectrophoretical alignement of microcapsules with encapsulating neurotrophic factor, which can provide physical, spatial and biological cues to modulate microenvironment of hydrogel for guiding the neurite outgrowth and differentiation of neural stem cells. The biodegradable, biocompatible, and conductive porous PLGA microcapsules with reduced graphene oxide/ silk-poly-L-lysine layer-by-layer assembly encapsulating bioactive molecular are aligned within photo-curable hydrogel between two parallel transparent and conductive plates, i.e., indium tin oxide, with comb electrode to integrate dielectrophoretically aligned microcapsule and controlled spatial biochemical and mechanical properties into a multifunctional platform for electrically active tissue regeneration. Here, we successfully synthesized porous PLGA microcapsule and fabricated conductive PLGA microcapsule by LbL assembly. By adding polyethylenimine in the process of double emulsion, the porous structure of microcapsule can be obtained by osmotic pressure and result in the positive charge of the microcapsule surface. The conductive PLGA microcapsules was also successfully aligned within photo-curable hydrogel by dielectrophoresis with applying an AC signal (1 MHz, 10 Vpp). The formed hydrogel scaffold can be used for guiding electroactive cell growth and differentiation.

Session No.: P5-164

Characterization of Acidic Decellular Methods of Porcine Cornea Scaffolds Seeded with Corneal Keratocytes

Hungjun Lin[1], Ting-Wei Li[2], Ying-Chen Chen[3], Ping-Lun Jiang[2], Chon-Ho Yen[4], Ching-Fu Tu[4], Ming-Thau Sheu[3], Der-Zen Liu[5]

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Damage to the cornea is one of the main reasons of blindness. Due to lack of supply of donor corneas, it is urgent to develop repairing or replacing bioengineered corneal scaffolds in order to restore normal vision. Decellular corneal xenografts are thought to be great human corneas substitutes for their natural extracellular matrix and biologic factors. The objective of this research was to establish promising decellular porcine corneal scaffolds, and recellularize by corneal keratocytes for restoring corneal biofunction. The porcine corneas were soaking in formic acid solutions at 25℃ for 24 h on an orbital shaker. Then decellular porcine corneal scaffolds were examined by scanning electronic microscope and transmission electronic microscope, and showed uniform parallel microstructure. DNA qualification assay indicated formic acid decellular method was successfully removed most cells inside the corneal scaffolds. GAGs and Collagen assay showed no difference between native porcine corneas and decellular corneal scaffolds, indicated well preserved extracellular matrix and major biologic factors. The decellular scaffolds were seeded with rabbit keratocytes, SIRC cells, and supported keratocytes growth for 1 week and 2 weeks. DAPI staining confirmed keratocytes well distributed inside the decellular corneal scaffolds. According to these results, the decellular corneal scaffolds have great basic mechanic properties and biologic properties, further in vivo tests should be investigated for corneal tissue engineering applications.

Session No.: P5-165

Fabrication and Characteristic of Yttria-stabilized Zirconia Using Negative Thermo-responsive Hydrogel

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The Yttria-stabilized zirconia not only the most commonly used ion conductor, and in the field of biomedical device and cutting tool which is widely used, because of high strength, toughness, and low modulus of elasticity. Such as artificial bones, joints, tooth root(implant). However, the powder sintering densification often requires forming by Cold isostatic pressing, or directly by hot isostatic pressing method. The purpose of this study was to investigate and analyze sintering properties of the novel ceramic --reverse thermo-responsive hydrogels combine with Yttria-stabilized zirconia powder (hydrogel mixture slurry/RTRH-3YTZP). This material can be regarded as functioning in a manner similar to the cold isostatic press (CIP) step before the powder sintering densification process. Finally, sintering densification is expected via free volume contraction, which will increase the mechanical properties. Method: Feasibility measuring and evaluate the best parameters and clinical applications of novel ceramic(n=10) by calculated density, Weibull modulus, Vickers hardness, Flexural Strength, fracture toughness values, and indentation fracture load was examined to deduce the optimum conditions of the IF method(IFM). As a result of this experiment, the average density was 5.02 g / cm3, flexural strength was 160.02 MPa (Maximum loading force = 129.01 N), while Weibull modulus was 10.36, the KIC values of group 2 were obtained: 7.80, 7.54, 7.39 MPam1/2, hardness (Hv) were group 2 better than others(471.61, 487.27, 337.68 kgf / mm2). Conclusions: The density value of group 2 (containing 10% hydrogel) has been close to the theoretical density (6.06 g / cm3), and the bending strength is still higher for the second group, and has a superior to natural teeth (anterior teeth and premolars bite force). In addition, Weibull modulus, fracture toughness and hardness values have the same results. Following conclusion is made: The second group result has applied to the medical field advantage.

Session No.: P5-166

Gradient Aligned Magnetic Nanocarriers in Gelatin-Silk Nerve Conduit

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Guidance cue, an important issue for nerve cell regrowth, which greatly affect axon elongation behavior. Here, we report the gelatin-silk nerve conduit (GSNC) integrating aligned NGF-encapsulated amphiphilic gelatin nanocapsules (N-AGNCs) that can guide nerve cell regrowth and continuously release nutrition. The AGNCs comprising superparamagnetic nanoparticles (SPIOs) and amphiphilic gelatin were synthesized by double emulsion, which exhibited excellent saturation magnetization (Ms) and a diameter of 250 nm. The N-AGNCs were incorporated into the GSNC as a precursor, and aligned via applying an external magnetic field before starting gelation in low temperature. To note, a gradient distribution of N-AGNCs can be constructed by adding AGNCs with different SPIOs concentration (e.g., 3-6 mg ml-1, which yields the Ms from 40 to 60 emu g-1) in GSNC. This gradient distribution of N-AGNCs can not only provide nutrition but guide the cell growth by arranging the distribution of NGF. Moreover, the degradation rate of the GSNC can be manipulated by adding different content of silk (i.e., 4.5 mg to 13.5 mg), and the gelatin (15 mg to 25 mg) can improve cell adhesion. Combining these features, the novel GSNC serves as an excellent cell culture platform for long-term cell observation, proliferation and guidance.

Session No.: P5-167

Antibiotic/Antifungal Drug-loaded Asymmetrically Porous Membrane /3D-printed Mesh Hybrid Tube as a Trachea Substitute

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Reconstruction of the damaged trachea caused by tumors, tracheal stenosis, congenital disorders, prolonged endotracheal intubation, and other types of tracheal trauma is one of the most difficult procedures in otolaryngology surgery. In this study, we prepared an asymmetrically porous polycaprolactone (PCL) membrane/3D-printed mesh hybrid tube loaded with dual antibiotic and antifungal drugs (amphotericin b and imipenem) for the damaged trachea treatment. The drug release behavior, mechanical properties, cytotoxicity, and antibacterial/antifungal effects of the hybrid tube were investigated. In vivo animal study to evaluate its remedial value for the trachea was also conducted using a rabbit model. The hybrid tube showed continuous drug release over 7 weeks (critical period for the effective treatment of trachea) and showed highly effective antibacterial effect during the experimental periods. From the animal study, it was observed that the dual drug-loaded hybrid tube had highly effective antibacterial effect and appropriately performed as a trachea substitute. From the results, we could suggest that the dual antibiotic and antifungal drug-releasing tube may be a promising therapeutic tool for the treatment of damaged trachea.

Session No.: P5-168

Production of 3D Porous Alginate with Rapid Prototype Binding Electrospun Chitosan / Pva Nano-fibrous Composite Materials

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Rapid Prototyping technology produce porous scaffold with interconnecting.It is benefit cell growth, nutrient transport and metabolism were excluded. Electrospun silk method produce large surface area and pore structure, in order to mimic the extracellular matrix. Then combined with rapid prototyping technology and electrospun technology to manufacture skin dressing composites. Electrospun material selection chitosan and polyvinyl alcohol are all biodegradable material, and use concentrated acetic acid (90vol%) as a solvent.It can effectively reduce the surface tension，increase the charge density, and improve mechanical properties by glutaraldehyde vapor cross-linked.It is regarded as the skin surface.Rapid prototyping material choice alginate with calcium chloride crosslinked to form gelled, .It is regarded as the dermal layer. And finally bonding two kinds of samples biological with glue to form composite. It was observed cross-sectional and surface of the composite with a scanning electron microscope.It is tested for tensile strength, degradation rate, moisture permeability. Composite skin moisture permeability value higher than normal and lower than the value of non-breathable container lid ， the tensile strength of the composite material is higher than the material rapid prototyping only, the degradation rate is lower than the value of rapid prototyping only.

Session No.: P5-169

Cyclic Stretching on the Growth and Morphology of Fibroblasts Embedded in High α-L-guluronic Alginate Hydrogel

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In biological tissue engineering, use the porosity three-dimensional structure to fill the vacant portion by hydrogel, to provide effective mobile both cell and nutrition. It is important that scaffolds require porosity, biocompatibility and degradability. Alginate is a not only non-toxic but water-soluble natural polymer material to fabricate scaffold. It can uniform mix with cell and provide growth environment, however due to low mechanical strength than alginate has fast degradation rate and tend to disintegration by external forces, no long time to maintain it shape; Relative to the high mechanical strength the external forces will help cells to stretching and promote cell proliferation. In the study, we used high mechanical strength alginate hydrogel to test, and using rapid prototyping systems made of porous scaffold; experimental compare stretching and non-stretching alginate hydrogel about cell growth、cell morphology、cell viability are discussed.

Session No.: P5-170

Ti6Al4V Titanium Alloy Surface Coating of Chemically Modified Chitosan Can Be Manufactured with Antibacterial Effect of on Osteoblast Culture

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Since titanium has good biocompatibility, widely used in clinical artificial tooth root, artificial joints, fractures, orthodontics, vascular stents and other applications, a broad antibacterial HHC10 antimicrobial peptides will interact with the bacterial membranes to destroy bacteria membrane, HHC10 peptides that will work with certain cell surface binding promote cell attachment, growth and differentiation. In this study, titanium alloy Ti6Al4V surface modification coating HHC10 had chitosan, through chemical cross-linking method of antimicrobial peptides HHC10 successful connected to the chitosan surface and antibacterial and biocompatibility testing from antibacterial test results that HHC10 surface modification had chitosan has an antibacterial effect, his study samples to 7F2 osteoblasts biocompatibility tests will be conducted to test the collagen, alkaline phosphatase activity and calcium mineralization test Learn differentiation test osteoblast, and cell proliferation, we can quantitatively by the DNA to the cell surface that chitosan modified after growth has increased over time, alkaline phosphatase and type I collagen in the first week promote differentiation has showed that we successfully synthesized peptide to the surface of chitosan, antibacterial and cell growth may reach environmental conditions.

Session No.: P5-171

Treatment of Atopic Dermatitis Using Drug-loaded Pva/Alginate Hydrogel

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that usually begins in infants or childhood, characterized by intractable pruritus, xeroderma, and relapsing eczematous skin lesions. For the treatment of AD, a variety of products are being developed such as creams, lotions, salves and sprays. However, the majority of the product is rapidly disappeared within 15 minutes owing to permeation and evaporation from the skin. In this study, we prepared prednisolone (drug usually used in the AD treatment) loaded-hydrogel of polyvinyl alcohol (PVA) embedded in mildly cross-linked alginate (PVA/mcALG hydrogel). The hydrogel can provide a moisturizing effect when applied on the skin, and form a film after one hour of skin coating and thus is easily detachable. We evaluated the anti-AD effect of prednisolone-loaded PVA/mcALG hydrogel for 4 weeks using a dinitrochlorobenzene (DNCB)-induced BALB/c mice model. The ear thickness and scratching behavior using hindlimb were investigated once a week. After 4 weeks of the treatment (on the final day of the experiment), level of immunoglobulin E (IgE) in serum were determined by ELISA. Also, skin samples from each mouse were recovered and stained with hematoxylin and eosin (H&E) and toluidine blue. And then, epidermal thickness and the number of mast cells in the skin layer of mice were measured. The prednisolone-loaded PVA/mcALG hydrogel treatment reduced ear thickness, scratching behavior, IgE levels and epidermal thickness as well as the number of mast cells. From the results, we could suggest that the prednisolone-loaded PVA/mcALG hydrogel may be a promising effective therapeutic system for the treatment of AD.

Session No.: P5-172

Treatment of Osteomyelitis and Bone Regeneration Using Dual Antibiotic/Growth Factor-loaded Alginate/Ha Hydrogel

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Osteomyelitis can result from trauma, nosocomial infection, or orthopaedic operation. Although the understanding of infectious diseases and the development of surgical/pharmaceutical therapeutic techniques are rapidly growing, osteomyelitis continues to be a challenge for orthopedic surgeons. Local delivery of antibiotics offers several advantages, including fewer side effects and higher local concentration of a given medicine compared to systemic one. However, the burst release of commonly used antibiotics due to their water-soluble property has been considered as a practical limitation for local delivery. Compared to the other biodegradable polymers, the in situ algi-nate/hyaluronic acid hydrogel possess several additional advantages, including easy preparation and free of the harmful organic solvents in the formulation process, etc. In this study, we prepared in-jectable antibiotic and/or growth factor [vancomycin and/or bone morphogenetic protein-2 (BMP-2)]-loaded alginate/hyaluronic acid hydrogels for osteomyelitis treatment and bone regenera-tion. The in vitro antibiotic and BMP-2 release, cytotoxicity, and antibacterial activity of the antibi-otic and BMP-2-loaded hydrogel were investigated. The in vivo study using rats was also conducted by Masson's trichorome and H&E staining, micro-CT analysis, blood analysis, biomechanical testing, and microbiological analysis. The dual vancomycin and BMP-2-loaded alginate/hyaluronic acid hydrogel showed more effective antibacterial and bone regeneration behavior than the hydrogels without them.

Session No.: P5-173

The Application of Thermo and Super-paramagnetic Responsive Polypeptide Hydrogels in Bone Tissue Engineering

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The suitable extracellular substrate can stimulate bone regeneration in bone tissue engineering (BTE), including ceramic nanoparticles (hydroxyapatite, HAP), but they are always having some limitations with size, morphology, surface roughness, dose dependent effect, and so on. These nanomaterials cannot be direct used to stimulate the growth and regeneration of bone. Therefore, this work attempted to solve these deficiencies. Nano-HAP coated with magnetic nanoparticles were used a chemical co-precipitation method. In the past, low frequency pulsed electromagnetic field (EMF) has been used in clinical application to reduce pain and inflammatory reaction induced by osteoarthritis (OA). Furthermore, nano-HAP coated magnetic nanoparticles could be applied with EMF to repair bone tissue. So, to our best knowledge, nano-HAP-coated magnetic nanocomposite (m-HAP) particles doped into polypeptide hydrogel have rarely been used in BTE scaffolds so far. In this study, we want to dope m-HAP into the polypeptide hydrogel, it can strengthen the magnetic effect in the EMF and increase the mechanical strength of the scaffold to promote hregeneration of cartilage and bone tissues.

Session No.: P5-174

Polydopamine-coated Uniform Polydimethylsiloxane Beads and Their Potential Biomedical Applications

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Many biocompatible constructs have been used to regenerate or restore function of damaged tissue. In this work, polydimethylsiloxane (PDMS) as a bioinert polymer was employed for the use of filler material. Due to its intrinsic hydrophobicity and poor cell adhesion ability, PDMS was coated with Polydopamine(PDA) to provide cell adhesive environment. Uniform sized PDMS beads were fabricated in oil-in-water emulsion system. Size of PDMS beads were controllable with changing discontinuous and continuous phase flowrate and concentration of PDMS solution. Produced PDMS beads were immersed in dopamine precursor solution and gently stirred with orbital shaker for 10 hours. Polydopamine(PDA) coated PDMS beads exhibited rough and dark surface, and low water contact angle. Cell adhesive ability of Pristine PDMS and PDA-coated PDMS was compared on cell culture experiment in sheet and bead type. As a result, PDA-coated PDMS beads exhibited less hydrophobicity and more cell adhesive property. With tunable size, bioinert, and cell adhesion property, PDA-coated PDMS beads have great potential for biomedical filler applications.

Session No.: P5-175

Thiourea/Sodium Periodate-Induced Rapid Formation of Polydopamine Coatings Under Acidic Conditions

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Dopamine has been widely used as a simple building block for surface modification of virtually any material surfaces. Until now, a few methods have been developed for increasing the deposition rate of polydopamine (PDA) coatings, such as electrochemically driven deposition, UV irradiation, and the utilization of strong oxidants. Although the above mentioned methods have been proved that they can even trigger the polymerization of dopamine under weak acidic conditions, the deposition rate is very slow, especially compared with their satisfactory performances in neutral or alkaline solutions. In addition, long-time immersion in the strong oxidants solutions may be harmful to some oxidant sensitive materials. Also some substrates are not suitable for modification by dopamine under alkaline conditions, such as pH sensitive gel, pH sensitive filter membrane or other alkaline corrosive materials. Therefore, looking for a method for the rapid formation of PDA coatings under acidic conditions still is a tremendous challenge. In order to solve this problem, N,N'-dimethylthiourea/sodium periodate system was used to induce the rapid formation of polydopamine coatings at acidic pH. Unlike the amino groups (pKa ~10) that are protonated under acidic conditions, the thiourea (pKa ~-1) groups are reactive towards the catechol derivatives under acidic conditions due to the low pKa value. So the thiourea group can act as an excellent nucleophile to react with quinone via Michael addition. The deposition rate of PDA coatings through this method is about 44 nm per hour, even comparable to the PDA deposition rate induced by strong oxidants at basic pH, while the sodium periodate-induced deposition rate is only about 1.3 nm per hour at the same acidic condition. Also, the N,N'-dimethylthiourea/sodium periodate-induced PDA coatings show high uniformity, enhanced chemical stability and good cell biocompatibility.

Session No.: P5-176

Using Polyplex to Enhance Gene Transfection in Cells

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The aim of this study was to develop polyplex for gene delivery. Two kinds of polymers, chitosan (CS) and polyacrylate (ERS), were included which composed of primary amino group and quaternary ammonium group in the monomer unit, respectively. The chitosan after deacetylated and depolymerized (DADPCS) was also included to investigate the effect of polymer molecular weight on gene delivery. These positive-charged polymers were used to complex with negative-charged plasmid DNA to form polyplex at various polymer/DNA (P/D) weight ratios via polyelectrolyte complexation method. The particle size of most polyplex was in the range of 134.5 ± 11.3-244.6 ± 1.8 nm with narrow size distribution (PDI < 0.2). There was no change in DNA B-type conformation after complexation by polymers, and the DNA was protected by polyplex without digestion by DNase I. No severe aggregation or dissociation of DNA polyplex was observed during storage at 4C for 28 days. Most of polyplex appeared low cytotoxicity, and there were at least 80% cells viable. The DADPCS-DNA-NP and ERS-DNA-NP polyplex significantly enhanced DNA transfection as compared to naked DNA. Nevertheless, the enhancement of DNA transfection by CS-DNA-NP was limited. Furthermore, increase of polymer/DNA weight ratio increased polyplex transfection as well. All of these results implied that the polymer type as well as molecular weight and the polymer/DNA weight ratio played important roles in dominating polyplex transfection.

Session No.: P5-177

Ph-sensitive Gold Nanoparticles Loaded in Mesenchymal Stem Cells for Photothermal Cancer Therapy

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Gold nanoparticles (AuNPs) have been widely investigated as a photothermal agent for cancer treatment because AuNPs generate localized heat upon near-infrared irradiation. However, increasing their tumor-targeting efficiency and maximizing photothermal effect by controlling the nanoparticle size still remain problems to be solved. In this study, we combine pH-sensitive gold nanoparticles (PSAuNPs) and tumor-tropic mesenchymal stem cells (MSCs) for effective photothermal cancer therapy. PSAuNPs loaded in MSCs are aggregated in mildly acidic endosomes, which increases the cellular retention of nanoparticles by preventing their exocytosis and enhances the photothermal effect by extending surface plasmon resonance effect. PSAuNP-laden MSCs (MSC-PSAuNPs) are actively migrated into tumor tissues, which improves the tumor-targeting efficiency and intratumoral distribution of these nanostructures. Compared to pH-insensitive, control AuNPs (cAuNPs), PSAuNPs show a 4-fold higher retention within MSCs. MSC-PSAuNPs that intravenously injected to mouse tumor model show a 37-fold higher tumor-targeting efficiency (5.6 % of the injected dose) and 8.3 ℃ higher heat generation compared to injections of cAuNPs after irradiation, which results in a significantly enhanced anticancer effect.

Session No.: P5-178

A Simple and General Method Using Electrospray Technique to Prepare Levamisole-Encapulating PMMA /PVP Microparticles: An in Vitro Study of Levamisole to Ovarian Cancer Cells

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Levamisole-encapsulating Poly(methyl methacrylate) and Poly(vinyl pyrrolidone) (LVM-encapsulating PMMA/PVP) micro particles were produced by the electrospray technique. A biocompatible material, PMMA, was mixed well with water soluble polymer, PVP, to generate the carrier for LVM. With the adjunction of PVP, it can render the water solubility for this drug delivery system. Microparticles were characterized by scanning electron microscopy (SEM) and Fourier transform infrared spectroscopy (FTIR) studies. SEM studies indicated the morphology of micro-particles in the size of around 1.5-2 μm. FTIR proved that Levamisole is encapsulated in microparticles successfully. In vitro studies, such as MTT assay on CP70 and SKOV-3 ovarian cell lines showed the anti-proliferative abilities of LVM- encapsulating PMMA/PVP particles.

Session No.: P5-179

Polyurethane Electrospun Nanofibers Containing Silver Nanoparticles: A Potential Nonwoven Mats for Antibacterial Wound Dressings

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Polyurethanes (PU) are frequently used as wound dressings due to its elastic properties and biocompatibility. Silver nanoparticles (AgNPs) have excellent antibacterial action against microorganisms and show good stability at ambient environment. In this study, AgNPs were incorporated into PU nanofibers and simultaneously generated the PU/Ag nonwoven mats. Silver nitrate (AgNO3) was reduced in ethylene glycol and redispersed in Tetrahydrofuran (THF). The PU was electrospinned into nanofibers using the electrospinning technique. The diameter of nanofibers are approximately 600~1000 nm and AgNPs are 5~30 nm in size characterized by scanning electron microscope (SEM). The antibacterial abilities of the nanofibers against Staphylococcus aureus strain was tested using the zone of inhibition method.

Session No.: P5-180

Application of Pig Model in Development of a Simple Mechanical Anastomosis Device

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This project was funded by grants from the technological innovation project, the Small and Medium Business Administration (SMBA) of Korea. Dentis Co., Ltd and Keimyung University Dongsan Medical Center have designed a prototype of an anastomotic ring device. We applied prototype devices manufactured in several different design in pig model that contain similar size of jugular vein compared to humans. To provide the efficient and reliable end-to-end vessels anastomosis, the device system that can be used for jugular veins was designed, fabricated and evaluated. We were assessed with biocompatibility, anastomotic leakage and functional outcome: angiographic imaging system, graft patency, foreign body reaction, clinical observation and histological analysis at Daegu-Gyeongbuk Medical Innovation Foundation-Laboratory Animal Center (DGMIF-LAC). In the anastomosis site, the blood leakage was not observed. None of the anastomosed vessels showed signs of early thrombosis. Examination of the anastomoses from the intimal side, showed no evidence of penetration into the lumen of the vessel. The anastomosis time was 3.9 ± 0.9 minutes (n=5) for ring device closure and 12.0 ± 6.6 minutes for suture closure. All 5 vessels were patent on clinical evaluation half an hour after completing the anastomosis. Anastomosis after 1 week, the medical ultrasound device (Sonoace7, Samsung Medison) was used for sonographic imaging data for evaluating vascular patency. The blood-flow has normally passed through the anastomosed vessels. The vascular angiographic images were acquired using biplane angiography (Artis-zee, Siemens) in Medical Device Development Center, Daegu-Gyeongbuk Medical Innovation Foundation (DGMIF). As a result, end-to-end vascular anastomosis performed with the ring device resulted in considerably faster reconstruction times when compared with traditional suture closure. Furthermore, the device is expected to use simply and safely in medical industry.

Session No.: P5-181

Customized Performance Evaluation for a New Developed Fecal Diverting Device to Protect Anastomotic Leakage at Dgmif-Lac

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Anastomotic leakage has been known as one of the most serious complications after bowel surgery, especially in low rectal anastomosis. The most common reason for a subsequent anastomotic leakage after the surgery is residual fecal matter. Therefore, in order for fecal diversion, a temporary diverting stoma is widely used to protect anastomotic area against further fecal contamination. Endovision Co., Ltd and Yeungnam University have developed a new type of defunctioning stoma from proximal colon above anastomosis to outside anus to protect rectal anastomosis. Daegu-Gyeongbuk Medical Innovation Foundation-Laboratory Animal Center (DGMIF-LAC) has assessed performance evaluation of the new fecal diverting device using beagle dogs supplied from Marshall Co. (Beijing). The device consists of two tire-like dumbbell-shaped outer balloons on the head portion which help to fix the device on the colon proximal to the anastomotic area without sutures. An absorbable mesh band was synthesized using Polyglycolic acid (PGA) to fix the head portion of the device externally on the colon. The dogs were monitored for 5 weeks to check body weight and defecation of dogs after anastomosis. We also evaluated anastomotic leakage and clinical examination utilizing x-ray imaging to site lesions. For 3 weeks, evaluation of the fecal diverting device had been performed until the device was expelled spontaneously. DGMIF-LAC is trying to make additional value creation by collaborative project and we have successfully carried out customized performance evaluation for a new developed medical device, which is the one of goals of the DGMIF-LAC.

Session No.: P5-182

Surface Modification of Titanium with Mao-Cap-Simvastatin Enhances Bone Formation

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In therapeutic bone repairs, autologous bone grafts, conventional or vascularized allografts, and biocompatible artificial bone substitutes all have their shortcomings. Tissue engineering may be an alternative for cranial bone repair. Titanium and it alloys are widely used in many clinical devices because of perfect biocompatibility, highly corrosion resistance and ideal physical properties. Statins, HMG-CoA reductase inhibitors, reduce cholesterol synthesis and prevent cardiovascular disease. Previous in vitro and in vivo studies showed that statin stimulated bone formation. In this study, we fabricated the titanium plate with dioxide creating by microarc oxidation and then electronic deposition of Ca.P that can carrier simvastatin to enhance large bone defect. Pure titanium plate (10×10×2 mm3) was made by 3D printing with pore size 600μm. The titanium plate was modified firstly with micro-arc oxidation (MAO) method. After MAO modification, Ca.P group (MAO-CaP) and simvastatin group (MAO-CaP-simvastatin) were using electrochemical deposition. The calvarial animal model was used New Zealand White rabbits to create a critical-sized defect (20-mm diameter). At 12 weeks post-implantation, rabbits were harvested. The torsion test was measured by torsion machine.The blood vessels were analyzed by micro CT. The bone formation rate was measure by SEM and quantification by Image-Pro Plus 5.0 software. In the results, the bone formation rate showed that MAO-CaP-simvastatin group was significantly higher than MAO and MAO-CaP at 12 weeks. Micro CT showed that at 12 week, all group showed that vessel growth into the central of the implant. Our data indicated that simvastatin can be carried on the surface of Ti after MAO method and electrodeposition and thus enhanced the now bone formation. Our study combined the concept of osteoinductive and osteoconductive to do the bone tissue regeneration.

Session No.: P5-183

Fabrication of Three-dimensional Cell Sheets by Molding Method

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A cell sheet, which is a tissue formed by development of cell-cell adhesion, is a promising technique for tissue engineering. Generally, the cell sheets consisting of multi cell layers were constructed by stacking of single layer sheet which is formed utilizing temperature-responsive culture plate. In this study, we established a direct formation technique of three-dimensional cell sheets by a molding method, as a new method without stacking process of cell layers. As a new scaffold for the construction of cell sheet, we prepared a culture mold which consisted of cylindrical chamber (4 mm in diameter and 1.5 mm in height) on polystyrene (PS) or poly-dimethylsiloxane (PDMS) substrates. The mold surfaces were modified with 2-methacryloyloxyethyl phosphorylcholine (MPC) molecules to create the cell non-adhesive surface. HepG2 cells formed spheroids (spherical cell aggregates) in the culture mold but did not form the cell sheet morphology when the cell density in the mold was low. However, in the high cell density culture, the cells gradually gathered within the mold and formed the cell sheet within 24 h of culture. The formed cell sheet had sufficient strength by development of cell-cell adhesion, and it could be collected easily from the mold. In the PS mold, the periphery regions of cell sheet consisted of viable cells, but dead cells appeared in the center region of sheet. In contrast, the occurrence of dead cells in the sheet was repressed in the PDMS mold which has characteristic of high gas permeability. Furthermore, the cell proliferation and functional expression of cell sheet formed in PDMS culture mold were higher than those in PS mold, indicating that PDMS mold is better material as culture scaffold. From these results, we have successfully established the new technique for the formation of three-dimensional cell sheets.

Session No.: P5-184

Enhanced Bone Repair Using Topographed Implant-induced Osteoblast Recruitment

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Enhanced Bone Repair using Topographed Implant-induced Osteoblast Recruitment Jeong-Kee Yoon,a Suk Ho Bhang,b Byung-Soo Kim,a,c,* a School of Chemical and Biological Engineering, Seoul National University, Seoul, Republic of Korea b School of Chemical Engineering, Sungkyunkwan University, Suwon, Republic of Korea c Bio-MAX Institute, Institute of Chemical Processes, Seoul National University, Seoul, Republic of Korea The regeneration of tissues initiates from the infiltration of host cells into the defect site. Thus, the rapid recruitment of osteoblasts is a prerequisite step for faster bone repair. We used physical guidance as topography that can direct and promote the migration of cells. In this study, we propose a topographically defined polymeric implant (TI) for the rapid recruitment of osteoblast into the tissue defect. The microgrooves with parallel arrangement (line), with radial arrangement (radial), and the non-patterned membrane (flat) was prepared as control, fabricated with Norland Optical Adhesive 86. The migration and proliferation of osteoblasts on the TIs were observed from in vitro study, and the intracellular molecular signaling was also studied to support the results. As the MC3T3-E1 cells migrated toward the empty area, the cells showed different migration profile on the 3 different types of TIs. The migration speed of the cells was faster on line and radial TIs compared to that of flat TI. Meanwhile, the radial TI exhibited faster coverage of defect site compared to line TI since the radial arrangement of microgrooves ‘focused’ cells more efficiently compared to parallel arrangement of microgrooves. These results are supported by PI3K, pAkt, Rac1 downstream signaling mechanism. The proliferation of the cells was also promoted on line and radial TIs, due to decrease of cell-cell contact. In in vivo study, radial TI showed the most efficient bone regeneration, in accordance to the in vitro results.

Session No.: P5-185

Development of a New Cell Separation Method Using Micropatterns

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When cells cultured on 3D scaffolds in which almost all of the sharp edges faced in same direction, cells migrated in one direction only. This phenomenon is a new form of cytotaxis induced by topological stimuli, and is a technology that is currently undergoing further development. In a previous study, we demonstrated that the subcellular shape of 3D patterned scaffolds affects cell migration [1]. To do this, we fabricated 3D micro-patterned scaffolds consisting of a scale structure incorporating equilateral triangular pores, a check structure incorporating regular tetragonal pores, and a stripe structure incorporating rectangular grooves. When then imaged the migration of NIH3T3 cells on the 3D scaffolds over 72 h. We found that the cells adhered to and extended along the edge of the upper surface of the 3D scaffold [2]. The angle at which the migrating cells turned differed markedly and correlated with the unit shape within the scaffold. We next identified a 3D shape that induced cells to migrate in only one direction. Three-dimensional scaffolds were constructed that bore sharp-edges, all of which faced in almost the same direction. NIH3T3 cells cultured on the 3D scaffolds migrated only in the direction faced by the sharp edges. We postulated that this phenomenon may be a new form of taxis induced by topological stimuli. We also partially identified the mechanism that caused the NIH3T3 cells cultured on these 3D scaffolds to migrate in only one direction. Confocal leaser scanning microscopy revealed that the sharp edges induced cells to put forth protrusions in a single direction. Thus, it appears that the 3D scaffold regulates the direction of these protrusions, thereby inducing unidirectional migration. I have now succeeded in separating different cells using this new technology. [1] SUNAMI et.al, e-JSSNT (2014). [2] SUNAMI et.al, Biomater. Sci. (2014)

Session No.: P5-186

Assembly of Three-dimensional Cell Sheet by Spheroid Fusion

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Formation of three-dimensional tissue, which is generated by aggregation of cell suspension, is a useful technique for tissue engineering, and the tissues termed cell sheet or spheroid are typical in vitro tissues. Here, our idea is to construct a larger tissue by utilizing the spheroid as building block. In this study, we generated a three-dimensional cell sheet by spheroid fusion using a microfabricated culture mold. To generate HepG2 spheroids, we used a microwell chip having the microwell diameter of 300 μm. This chip allowed the production of a large number of homogenous spheroids of approximately 120 μm in diameter. Next, the spheroids collected from the chips were re-seeded into a microfabricated culture mold, which had cylindrical chamber of 4 mm in diameter. Two similar culture molds, which made from poly-methylmethacrylate (PMMA) or poly-dimethylsiloxane (PDMS), were used to evaluate the formation of cell sheet. HepG2 spheroids seeded to the culture mold gradually gathered and fused with each other, and consequently the cell sheet was formed within 24h. The degree of spheroid fusion in the PDMS mold was higher than that in the PMMA mold. Furthermore, the cell proliferation and albumin secretion activity in the PDMS mold were higher than those in the PMMA mold. This difference may be caused by the gas permeability of mold material, and the PDMS mold which has high permeability of oxygen may affect the properties of cell sheet. These results indicate that the spheroid fusion using the PDMS culture mold is a promising technique for the cell sheet formation.

Session No.: P5-187

Electrochemical Corrosion and Biocompatibility of Porous Ti6Al4V Alloys Fabricated by Electron Beam Melting

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Porous titanium alloys have received extensive attention as the orthopedic implants duo to their excellent mechanical properties and three-dimensional structures similar to human bone. The recent application of electron beam melting (EBM) for the processing of titanium alloys led to one-step fabrication of customized porous titanium implants with complex geometrical features and controlled porosity. The purpose of this study is to test the electrochemical corrosion behavior and biocompatibility of porous Ti6Al4V alloys fabricated by EBM. Open circuit potential (OCP), potentiodynamic polarization and electrochemical impedance spectroscopy (EIS) tests were performed to study the corrosion resistance of EBM-produced porous Ti-6Al-4V alloys in phosphate buffered saline solution. Surface microstructural characterizations were utilized by scanning electron microscopy (SEM), X-ray diffraction (XRD) and transmission electron microscope (TEM). Electrochemical results show that EBM-produced sample possesses better corrosion resistance than the traditional wrought Ti6Al4V alloys, which is attributed to the even distribution of the alloying elements in the alpha and beta phase developed during the electron beam melting process. The cell culture assays reveal that the EBM-produced porous Ti6Al4V scaffolds not only show good biocompatibility, but also can improve the adhesion, growth and proliferation of MC3T3-E1 cells, indicating no adverse effects on cell functions. This study demonstrates that porous Ti6Al4V implants generated by electron beam melting have excellent prospects in orthopedic applications.

Session No.: P5-188

PCL Porous Beads with Leaf-stacked Structure for Sustained BMP-2 Release

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To reconstruct damaged tissues or organs, several strategies using cells, scaffolds, and bioactive molecules have been explored. In recent, a new approach, in situ tissue regeneration, has been introduced. This new concepts regulate human body's microenvironments with bioactive molecules to induce recruitment of host stem cells or progenitor cells to damaged site. The maintenance of host microenvironment relies on controlled and effective release of bioactive molecules. Bioactive molecules-loaded scaffolds which can allow controlled release of bioactive molecules as well as provide a frame for cell adhesion/growth/differentiation are considered as a first-line tool for target tissue/organ regeneration. To introduce the bioactive molecules on certain matrices, surface modifications based on physicochemical reaction/interaction are commonly utilized. However, the potential toxicity of chemical residues used for the surface modification is considered as a major huddle for clinical applications. Therefore, the main aim of this study was to develop a porous bead with leaf-stacked structure which can allow sustained release of bioactive molecules without any chemical modification. The morphology, BMP-2 release profile (using ELISA kit and rhodamine-conjugated BMP-2), and induction potential for osteogenic differentiation of periosteum-derived cells of the BMP-2-loaded porous bead were investigated.

Session No.: P5-189

Fabrication and Characterization of BMP-2-loaded GBR Membrane with Unique Morphology for New Bone Formation

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Sound healing of the large bone defect is one of the critical challenges in clinical fields, including oral, maxillofacial, orthopedic, and plastic surgery. In general, the rapid appearance of connective tissue in bone defect during healing which lead to the incomplete bone formation has been considered as a major limitation in bone regeneration. To solve this problem, guide bone regeneration (GBR) membranes which can prevent connective tissue infiltration into the bone defect, and nutrient penetration into bone defect, thus allow sound bone regeneration has been utilized in clinical settings. In this study, we prepared an unique GBR membrane with leaf-stacked structure which may meet the essential requirements of GBR membrane as well as allow sustained release of bioactive molecules (i.e., BMP-2). Their morphology, mechanical property, wettability, permeability, BMP-2 release profile, and the osteogenic differentiation potential of periosteum-derived cells were characterized. And also the new bone induction potential of the BMP-2-loaded GBR membrane was compared to the control (blank) and commercial products using SD rats (cranial defect model).

Session No.: P5-190

Reconstruction of Transvaginal Repair in the Pelvic Organ Prolapse via Functional Polypropylene Mesh

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Numerous modifications have been developed over the past two decades seeking to improve the transvaginal repair in the pelvic organ prolapse (POP) by using polypropylene (PP) mesh implants. The hydrophobicity of PP, however, presents a great hindrance for translating potential technologies into viable clinical applications. In this study, by manipulating self-polymerization and strong adhesive characteristics of dopamine, we developed a facile method to enhance the transvaginal repair by modifying PP meshes with polydopamine (PDA), which allows easy grafting of basic fibroblast growth factor (bFGF) onto the surface of PP. Such surface modification of PP meshes with bFGF was found to efficiently promote bioactivity without changing the morphology or mechanical properties of the PP meshes. Additionally, bFGF-modified PP meshes significantly promoted cell viability and adhesion compared to the unmodified PP. Ultimately, after three months implantation, the bFGF-modified PP meshes exhibited improved tissue repair with greater degree of organization of deposited collagen, increased tensile strength and reduced inflammatory response. Overall, the surface-modified PP meshes will be highly practical as templates for transvaginal repair in the POP treatment.

Session No.: P5-191

Application of Supercritical Carbon Dioxide in Preparation of Porcine Skin for Tissue Engineering: Natural Collagen Scaffold as a Wound Dressing

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Background: The extracellular matrix (ECM) of animal origin has been extensively used in the field of tissue engineering and regenerative medicine. Decellularization of tissues or organs by chemical and enzymatic methods have been used to remove endogenous cellular components from xenografts. These methods can introduce process residues that can cause bioincompatibility and increase the likelihood of host tissue immune responses. To circumvent these problems, a novel supercritical carbon dioxide (SCCO2) method was used to process an unadulterated porcine dermis into an acellular matrix that may function as a wound dressing. Methods and Results: Dermal layer was physically detached from the porcine hide. Tissues were then immersed in NaOH and H2O2 solutions, followed by SCCO2 extraction to complete decellularization. Decellularization was evaluated histologically via hematoxylin-eosin (H&E) and 4', 6-diamidino-2-phenylindole (DAPI) staining. The structure of the collagen scaffold was examined by scanning electronic microscopy (SEM). No cells remained on the scaffold after decellularization. The porous dermal collagen structure stayed intact. In vitro results indicated that the fibroblast cells were able to migrate into the decellularized scaffold and proliferate. Animal wound healing model showed that the decellularized dermal scaffold has better preclinical efficacy than a commercially available product. Conclusions: As a proof of concept, SCCO2 successfully processed dermal tissues into a potentially clinically effective wound dressing device. These results can provide valuable fundamental information that can support future designs and fabrications of other biomaterials for medical applications.

Session No.: P5-192

In Vivo Bone Regeneration of Thai Silk Fibroin-based Scaffolds with Gelatin, Hydroxyapatite and Hyaluronic Acid

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Thai silk fibroin-based scaffolds with gelatin conjugation and hydroxyapatite depositing have been developed and reported on their appropriate physical properties and promising potential to promote the growth of bone cells in vitro in our previous study. In addition, they were shown to be non-toxic to cells in vivo. In this work, three types of Thai silk fibroin-based scaffolds, including conjugated gelatin/Thai silk fibroin scaffold (CGSF), hydroxyapatite/conjugated gelatin/Thai silk fibroin scaffolds (CGSF4) and hyaluronic acid/Thai silk fibroin (HSF), were investigated for their in vivo bone regeneration potential in rat model. Each Thai silk fibroin-based scaffold was implanted in the bone defect (6 mm) on the radius bone of Wistar rat. After 12-week of implantation, bone regeneration was analyzed by micro-CT and the semi-quantitative data evaluated from histological slides, compared to the control group (no implanted scaffold). From micro-CT results, new bone was formed at the cutting end of the defect site of all groups. Interestingly, there was new bone formed in the middle of scaffolds, but not in the control group. In particular, the most remarkable new bone formed was noticed in the implant case using CGSF4 scaffold. As a result, Thai silk fibroin scaffold modified with gelatin conjugation and hydroxyapatite deposition (CGSF4) possessed a great potential to be employed as bone scaffold for bone tissue engineering application.

Session No.: P5-193

Preparation and Evaluation of Silk Fibroin/Cartilage Extracellular Matrix Composite Oriented Cartilage Scaffold

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Regeneration of articular cartilage remains a formidable challenge in clinic due to the complexity and limited self-repair capability of native cartilage tissue. Cartilage tissue engineering is a promising strategy for the treatment of articular cartilage defects. In view of the advantages and disadvantages of numerous scaffold materials, this study mixed the modified acellular prepared porcine articular cartilage extracellular matrix (CECM) up with silk fibroin (SF) as cartilage scaffold materials. Oriented scaffolds were fabricated by proportional mixing ultra-unidirectional solidification as a freezing process and lyophilization technology. The porcine articular CECM scaffold was developed as a control group by the same method. The SF-CECM composite oriented scaffolds and the CECM oriented scaffolds were cross-linked by 1-ethyl-3-3dimethyl aminopropyl carbodiimide (EDAC) and N-hydroxysuccinimide (NHS). The physical and chemical properties and biocompatibility of the SF-CECM composite oriented scaffolds and CECM oriented scaffolds were evaluated. To the two groups, the pore structure and the pore size were well, the water absorption was above 95%, and the pore connectivity was well, which accorded with the requirements of cartilage tissue engineering. Orientation structure succeeded to maintain the mechanical properties of articular cartilage, and the compressive elastic modulus of the composite group was higher than that of the control group. SF-CECM composite scaffolds and CECM oriented scaffolds had no cytotoxicity and no effect on cell proliferation; Scanning electron microscope revealed that the adipose-derived stem cells grew and secreted extracellular matrix in the scaffold; H/E staining showed that the cells were evenly distributed in the pores of the scaffolds. The results of this study confirm that SF-CECM composite oriented scaffold is a desirable scaffold for cartilage tissue engineering, which provided a new method and idea for cartilage tissue engineering.

Session No.: P5-194

Development of Artificial Tears Solution with Anti-Inflammation Component for Dry Eye Syndrome Treatment

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Dry eye syndrome (DES) is a general disease in ophthalmic clinic causing by ocular surface inflammation. Artificial tear (AT) is used to treat DES, but it's only relieve the symptoms, and non-effective. This study assessed the anti-inflammatory effect of AT containing epigallocatechin gallate (EGCG) and hyaluronic acid (HA) for treating DES. Human corneal epithelial cells (HCEC) were used to determine the safe dose of EGCG by WST-8 assay. Lipopolysaccharide-stimulated HCEC showing inflammation were treated with EGCG/HA combination. The expression of genes IL-1β, IL-6, IL-8, and TNFα was confirmed using real-time PCR. Physical properties such as viscosity/osmolarity of the AT were examined. The AT containing EGCG and HA was topically administered in a rabbit DES model established by treating with 0.1% benzalkonium chloride (BAC). Tear secretion, fluorescein, H&E/TUNEL staining and inflammatory cytokines of corneas were examined. Non-toxic concentration of EGCG for HCECs cultivation was 10 μg/mL. Inflamed HCECs treated at 10 μg/mL EGCG and 0.1% HA (E10/HA) showed significant inhibition of inflammatory genes: IL-1β, IL-6, IL-8, and TNFα than in treated with EGCG or HA separately. The AT contained E10/HA mimicked human tears, with similar osmolarity and viscosity. HA addition increased drug retention on the ocular surface. Topical treatment with AT plus E10/HA in DES rabbits can increased tear secretion, reduced corneal epithelial damage, maintained epithelial layer and stromal structure. Moreover, the corneas of E10/HA-treated group showed less apoptotic cells and lower inflammation condition. In conclusion, the efficiency of the AT containing 10 μg/ml EGCG and 0.1% HA as a topical agent with anti-inflammatory and mucoadhesive properties for effective treating DES rabbits was proofed.

Session No.: P6-195

Staphylococcal Enterotoxin C2 Expedites Bone Consolidation in Distraction Osteogenesis

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Distraction osteogenesis (DO) technique could be used to manage large-size bone defect sucessfully, but DO process usually requires along duration of bone consolidation. Innovative approaches for augmenting bone consolidation in DO is of great need. Staphylococcal enterotoxin C2 (SEC2) has been developed and found to suppress osteoclastogenesis of mesenchymal stem cells in vitro. In this study, we investigated the effect of SEC2 on proliferation and osteogenic differentiation of rat bone marrow derived mesenchymal stem cells (rBMSCs). Further, we locally administrated SEC2 (10 ng/ml) or PBS into the distraction gap in Sprague-Dawley (SD) male rat DO model every three days till termination at 3 and 6 weeks. The distraction regenerates were subjected to X-rays, micro-computed tomography (μCT), and mechanical testing, histology and immunohischemistry examinations to assess new bone quality. SEC2 had no effect on cell viability. The calcium deposition was remarkably increased and the osteogenic marker genes were significantly up-regulated in the rBMSCs treated with SEC2. In the rat DO model, the SEC2 group had higher bone volume/total tissue volume in the regenerates. At 6 weeks, the mechanical properties were significantly higher in the SEC2-treated tibiae comparing to the control group. Histological analysis confirmed that the new bone had improved quality in the SEC2 treated group, where the osteocalcin and osterix expression in the regenerates was up-regulated, indicating faster bone formation. The current study demonstrated that SEC2 local injection promotes osteogenesis and enhanced bone consolidation in DO. The findings support application of SEC2 as a potential novel strategy to expedite bone consolidation in patients undergoing DO treatment.

Session No.: P6-196

Direct Cell Migration by Using an Air Plasma Roughened Substrate and Patterned Extra Cellular Matrix

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Studies have found that bond healing is a complex process, and it depends on the proliferation, migration and differentiation of cells. Among them, migration plays an important role to facilitate healing process. In current clinical method, a biodegradable or non-degradable physical barrier is usually surgically implant to limit the direction of cell migration for shortening healing process. However, it could become a long term stress that patients need to suffer. To understand the physiological cues that could promote cell migration, studies have shown that a roughened substrate could promote the migrating speed of bone cells, especially substrates with roughness higher than 1.5 μm. However, the direction of cell migration was still not controllable. In this study, we present our method on combining patterned extra cellular matrix and a roughened substrate for controlling both of direction and speed of cell migration. The designed substrate was a biocompatible polyvinylidene fluoride (PVDF) polymer treated by air plasma, where a large area of roughened surface could be created. Then, the surface was coated with 10 nm SiO2 with 20 nm Ti adhesive layer. Then, striated-pattern fibronectin islands were coated on the PVDF surface by standard polydimethylsiloxane (PDMS) contact printing method. Multiple 10 mm by 20 μm wide fibronectin islands with 20 μm separation were printed. Osteosarcoma MG-63 Cells were used as the cell model, and migratory pattern of cells were monitored for 12 hours. Experimental findings suggested that the cells could align and conform directionally to the patterned fibronectin islands. Further, cells migrate faster on air plasma treated surface at a migration speed of 2–10 μm/hour in a direction along with printed fibronectin. On the contrary, cells are nearly stationary on untreated and unpatterned substrates. Detailed experimental studies and characterizations will be presented in this paper.

Session No.: P6-197

The Biological Changes of Early Continuous Passive Motion and Treadmill Exercise for Articular Cartilage: A Non-anterior Cruciate Ligament Reconstruction Model in Rabbits

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It still remains controversial, especially when taking into consideration the risk of osteoarthritis (OA), whether receiving an early reconstructive surgery post acute anterior cruciate ligament (ACL) rupture is recommended or not. Alternatively, a suitable exercise offers stimulus for reducing joint inflammation and facilitating cartilage regeneration. Continuous passive motion (CPM) and active treadmill exercise (TRE) have been shown to increase cartilage repair. However, the biological changes of CPM with or without TRE in the early ACL non-reconstruction stage remains unclear. The purpose of this study was to understand the effects of early CPM with or without TRE after ACL rupture. It was hypothesized that non-loading CPM could offer a short-term protective effect for cartilage in the damaged joint, but TRE may attenuate the effect due to the joint's mechanical instability. Thirty adult New Zealand White male rabbits were studied and randomly assigned to four groups: the Rest group (sedentary), CPM group, TRE group and CPM+TRE group. Each rabbit had an ACL transection (ACLT) performed on the right knee and, for the control, on the contralateral knee. The knees were evaluated at 4 weeks after the surgery for gross appearance, quantitative OA scores and histological examination. The CPM group showed the best protective therapeutic effects. In gross appearance, the CPM group displayed delineated normal articular surfaces, but the Rest and TRE groups demonstrated surface abrasion. The total OA scores in the TRE group (13.14) were significantly higher than the CPM+TRE (10.38) and CPM (7.88) groups. Histological examination showed that the CPM group manifested sound chondrocyte arrangement and abundant glycosaminoglycan (GAG) content, while the Rest and TRE groups did not. These results suggest that CPM after acute ACL injury for short-term articular cartilage protection is beneficial, while TRE should be judiciously applied.

Session No.: P6-198

Influence of Mechanical Stimulation on the Annulus Fibrosus Cells Seeded on Circumferentially Oriented Microfiber Scaffold Prepared by Wet-spinning

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Repairing damaged annulus fibrosus (AF) is challenging for treating intervertebral disc (IVD) disease. Tissue engineering may be a promising alternative. However, manufacturing scaffolds with circumferentially oriented fiberous structure similar to native AF still remains a challenge, and mechanical stimulation on the behavior of cells seeded on the AF scaffolds remain unknown. In this study, we present a straightforward and practical strategy for fabricating an AF scaffold with circumferentially oriented poly(ε-caprolactone) or AF microfibers. The influence of dynamic mechanical stimulation on the annulus fibrosus cells (AFC) seeded on oriented silk scaffolds(SFS) was also investigated. The architecture of the scaffold was characterized by SEM. AF cell culture demonstrated that this scaffold could support AF cell attachment, proliferation and infiltration, as confirmed by SEM, live/dead staining, and MTT assay. Histological, immunohistochemical staining, biochemical quantitative analysis and RT-PCR showed that the AF cells inside scaffolds could secrete AF-related extracellular matrix. Moreover, the AF-related ECM was circumferentially oriented along the microfiber direction. The compressive and tensile properties were enhanced with increasing culture time. The new wet-spun microfibrous oriented scaffold has great potential as a substrate for regeneration of AF. Mechanical stimulation was imposed on the SFS seeded with AFC at a range of strain amplitudes (5, 10, 15, 20 %) for 2 hours per day for two weeks. The compressive elastic modulus reached to maximum value under 15% strain, while height of SFS was maintained unchanging. Histological and immunohistochemical staining and quantitative biochemical analyses were utilized to detect the changes in the biochemical properties. As a result, the collagen and GAG contents increased to maximum value under 10% and 15% strain respectively. This study demonstrates that mechanical stimulation could improve the ability of AFC to produce ECMs. The combination of SFS with mechanical stimulation provides a new strategy for tissue engineered AF.

Session No.: P6-199

Small-diameter Tissue Engineered Vascular Grafts Based on Decellularized Human Umbilical Arteries

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Atherosclerotic vascular diseases are the leading cause of morbidity and mortality in modern society. A variety of tissue-engineering strategies have thus been developed for functional small-diameter vascular grafts. The mechanical properties of a tissue-engineered vascular graft are critical for its success. This issue is less of a concern if decellularized arteries are used as a scaffolding material as decellularization preserves the majority of extracellular matrix and hence the mechanical properties of native arteries. Decellularized human umbilical vessels (HUA), particularly, represent a potential scaffolding material for small-diameter TEVGs because their dimensions are comparable to target arteries and sufficiently long grafts without branches are achievable. However, the dense adventitia could not only decrease the efficiency of decellularization but also the cell infiltrations during cell seeding procedure. In this study, we selectively removed the adventitia of HUAs via enzyme treatment. The treated HUAs were then cannulated into a custom-made bioreactor and perfused / pressurized with 1% hypotonic sodium dodecyl sulfate solution. After that, the decellularized HUAs were implanted into abdominal cavity or subcutaneous of rats for 1 week, 2 weeks, and 4 weeks. We found that the efficiency of decellularization was significantly increased after adventitia removed. Furthermore, the cell infiltrations were occurred at the first week of implantation in the adventitia removed group while the delayed cells infiltrations up to 4 weeks in the non-adventitia removed group. We conclude that the removing of adventitia could increase the efficiency of decellularization and cell infiltrations in HUAs.

Session No.: P6-200

Development of Cryopreservation Method for Polydactyly Derived Chondrocyte Sheets by Vitrification

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【Introduction】 We are currently preparing for a Japanese Ministry approved clinical study using allogeneic chondrocyte sheets derived from polydactyly patients. Polydactyly derived chondrocytes proliferate rapidly, and thus mass production can be realized; however, making chondrocyte sheets widely available requires proper storage systems. We have already reported an effective vitrification method for rabbit chondrocyte sheets (BMC Biotec. 2013). Here, we aimed to establish effective vitrification conditions for human polydactyly derived chondrocyte sheets.

【Methods】Under the approval and guidance of the Clinical Research Review Committee, cartilage tissues obtained from polydactyly patients (avg. 13.3 months) were used to create chondrocyte sheets. The sheets were pre-treated with equilibration and vitrification solutions, permeated with a cryoprotectant, packaged in aluminum, and then vitrified. The pre-treatment was set at 10, 20, 30, or 45 minutes to determine optimal conditions. To recover, vitrified sheets were placed on a heating plate (38℃) and transferred to a sucrose solution to diffuse out the cryoprotectants. Sheets were checked for cracks, and cell viability was determined by trypan blue staining. The production of cartilage anabolic factors (TGFβ1, MIA) by vitrified sheets were measured by ELISA and compared with that of non-vitrified controls.

【Results】 All sheets were visibly undamaged. The number of viable cells per sheet was the highest for 10 minutes pre-treatment and comparable to that of the control group (2.41±0.45x10^6 vs. 2.98± 0.69x10^6, n=8, p=0.54). TGFβ1 production (ng/ml) was equivalent to 86% of control group (2.44±0.37 vs. 2.82±0.60, n=7, p=0.07). MIA production (ng/ml) decreased significantly to 67% of control group (9.38±4.21 vs. 13.99±6.47, n=7, $p<0.05$).

【Conclusion】Vitrification of polydactyly derived chondrocyte sheets was possible. The production of anabolic factors likely depends on the number of viable cells; nevertheless, MIA production was reduced at a cellular level. We will further explore such issues and optimize the vitrification method for cell viability and functionality.

Session No.: P6-201

Evaluation of Anti-Inflammation Effect of Tea Polyphenol-Contained Nanoparticles for Dry Eye Syndrome Treatment

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Dry eye syndrome (DES) is a common disease causing by instable tear film induce ocular surface inflammation. In ophthalmological treatment, topical delivery of ocular therapeutics such as eye-drop is usually used, but ocular barrier of eyes can impede the release of pharmaceutical drugs, local bioavailability of the drug decreases. The main objective of the study is to develop a new nanomedicine for dry eyes syndrome treatment. Gelatin was adapted as the drug carrier with surface coating of hyaluronic acid (HA) to increase drug retention on the cornea. And the polyphenol, epigallocatechin gallate (EGCG), was encapsulated in the nanocarriers as the anti-inflammation agent to treat DES. These nanomedicine were abbreviated as GE and GEH, its particle size and zeta potential were about 142.1 ± 32.6 , 253.4 ± 7.3 nm and 23.2 ± 0.5 , 9.2 ± 1.8 mV. Benzalkonium chloride (BAC) at concentration of 0.1% was applied to the rabbit ocular surface for DES inducement. Then, the GE and GEH nanoparticles was used as an eye-drop to instilled in healthy and experimental dry eye rabbits. No ocular discomfort and irritation phenomenon were observed in rabbits after topical delivery of GEH formulation. In DES rabbits, both parameters significantly improved after GEH nanomedicine treatment. Anterior eye segment of treated rabbits showed normal architecture and morphology. Such as tear production and fluorescein staining. These results identify a potential application of EGCG-nanomedicine as a new therapeutic modality for the treatment of dry eye disease.

Keywords: Dry eye syndrome, Epigallocatechin gallate, Hyaluronic acid, Nanocarrier, eye-drop

Session No.: P6-202

Effect of β-Carotene/Silk Fibroin Film on Proliferation of Corneal Endothelial Cell

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We manufactured transparent, stable and insoluble silk films by blending with β-carotene as an alternative scaffold for bioengineering new cornea. The morphological and structural properties of films were analyzed using contact angle, field emission scanning electron microscope (FESEM) for film surface, MTT assay for cell proliferation, reverse transcription polymerase chain reaction (RT-PCR) for expression of mRNAs and histological analysis. Furthermore, In vitro biological compatibility was studied using rabbit corneal endothelial cells (rCEnCs) as models. β-carotene content affected in transparency of each β-carotene/silk fibroin films (β/SF), but it was not significant. The results showed that the β/SF film efficiently increases the rCEnCs adherence, growth and maintains cell morphology, formation of cell junctions and gene expression required for functional rCEnCs. Thus, the results suggest that silk fibroin offers good environment as carrier and β-carotene plays a role to improve biological properties. Overall results showed that β/SF can be used as a suitable alternative for high quality corneal tissue expansion and transplantation. This research was supported by NRF-2012M3A9C6050204.

Session No.: P6-203

Reduced Graphene Oxide Enhances Expression of Angiogenic Growth Factors and Gap Junction Protein of Mesenchymal Stem Cells for Cardiac Repair

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Myocardial infarction (MI) is one of the major cardiovascular diseases. Mesenchymal stem cell (MSC) implantation has been emerging strategy for cardiac repair after MI. Herein, we report reduced graphene oxide (RGO) which shows high affinity to extracellular matrix (ECM) proteins and high electrical conductivity. We hypothesized that RGO into MSC spheroid would improve the therapeutic efficacy for MI via the secretion of paracrine factors and the upregulation of gap junction proteins. RGO flakes incorporated into MSC spheroids by adding RGO flakes at concentrations of 0, 2.5, 5, and 10 μg mL-1 (Sph-0, 2.5, 5, 10, respectively) to MSC suspension. Sph-5 showed a balanced combination of cell-cell and cell-RGO interactions. Therefore, Sph-5 was chosen for further analysis. The expression of FAK/pFAK, ERK/pERK, VEGF in spheroids was analyzed by western blot. FAK/pFAK, ERK/pERK, VEGF were increased in Sph-5 compared to Sph-0, 2, 5, 10. The functionality of Cx43 was examined by dye transfer analysis with calein AM and DiI. The functionality test of Cx43 showed that the presence of functional gap junction in Sph-5. MI was induced in 8-week-old BALB/c nude mice. One week after MI, PBS, RGO flakes, Sph-0, or Sph-5 were injected into the border zone. Two weeks after cell injection, the hearts were excised and transversely. In mice MI model, Sph-5 showed high capillary density, low fibrosis area, and high Cx43 expression compared to other groups. In addition, the implantation of Sph-5 improved cardiac function. RGO could not only promote paracrine factors secretion and gap junction crosstalk, but also could be fabricated into implantable thin shape. RGO flakes could promote important factors of MSC spheroid for cardiac repair. Therefore, MSC-RGO hybrid spheroids can be used therapy for MI.

Session No.: P6-204

Cellular Response in Rabbit Osteoporotic and Healthy Bone for Intact and Sham Control in Biomaterial Implantation

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Due to the ethical and quantitative limitation limited number of animals are used for controls in biomaterial field. The most common controls represent intact bone, sham control (drilled hole without implantation) and maximally similar to the pathological condition experimental model for bone, for instance, osteoporosis. However, there is no strong evidence regarding the differences between molecular events in different controls used in implantology. Thus, our aim was the comparison of hard tissue reactions in different control bones to evaluate the real variations in distribution of molecular factors. Material and methods. We used 4 nonoperated and 6 sham New Zealand rabbits. For sham controls 2 mm channel in the bone was drilled and sutured. After 6 and 8 months euthanasia was performed. For the third group of 6 female rabbits who underwent ovarectomy, 1.5 months later the euthanasia was performed. Tissues were prepared for detection of OPG, NFkb105, MMP2, BMP2/4, IL1, and IL10 by use of immunocytochemistry. Results. Numerous to abundance of BMP2/4-, BMP2/4-, IL10-containing cells were seen in intact bone, moderate number of them characterized osteoporotic one, but sham control increased expression of all factors. OPG showed numerous positive osteocytes in intact bone, but equally decreased in osteoporotic and sham control. NFkb105 showed similar to the OPG expression along the controls, just for the higher numbers of cells. IL1 demonstrated only moderate number of cells in intact bone, than decreased in osteoporotic one and slightly elevated in sham controls. Conclusions. Osteoporotic bone demonstrates decreased growth, cellular activity, degradation, and also pro- and anti-inflammatory ability. Sham control bone renovates the growth, degradation, local immune status until the intact bone indices seemingly due to the traumatic induction. OPG/NFkb105 bone remodelation seems to be a stable self-regulating system seriously depending on the any epigenetic factors.

Session No.: P6-205

Effect of ANO5 on Osteoblast Differentiation

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Gnathodiaphyseal dysplasia (GDD; MIM#166260) is a rare skeletal disorder with autosomal dominant pattern and characterized by cemento-osseous swellings of jawbones and fragile long bones with diaphyseal sclerosis. The mutations in ANO5/TMEM16E gene which encodes a member of the calcium-activated chloride channel family, has been identified to be associated with GDD. In this study, we utilized the MC3T3-E1 pre-osteoblast cell lines (clone 14) to study effects of ANO5 on osteoblast differentiation. Knocking down the ANO5 gene with specific shRNA in MC3T3-E1 cell lines (clone 14) resulted insignificantly increased expression of osteoblast markers osteocalcin(20-fold), 1-collagen 1(up to 1.4-fold), Runx2(up to 2-fold)and osterix(up to 2-fold) messages in a stage-specific manner over a 21-day period compared to untransfected and scrambled controls. Alizarin red staining at culture days 14 and 21 indicated increased mineral nodule formation in Ano5 knock-down cultures. The expression of endogenous ANO5 protein in MC3T3-E1 cells increased consistently during a 21-day MC3T3 culture period. These evidences suggest that ANO5 play an important role in osteoblastogenesis as a negative regulator and we conclude that functional defect of ANO5 protein enhance osteoblast differentiation and possibly regulate bone matrix deposition. The effect of mutant ANO5 in osteoblasts may in part explain the diaphyseal hyperostosis, the severe bone resorption in the jaws and the replacement by soft fibro-osseous tissue with characteristic psammamatoid inclusions. In future, knockout mouse model should be taken into account to reveal the precise mechanism on how the mutant ANO5 results in the GDD phenotypes.

Session No.: P6-206

Effect of Duck's Feet Derived Collagen and Poly(Lactic-co–Glycolic Acid) Scaffold on Bone Regeneration in Rat Calvarial Defect Model

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Bone substitutes during bone defects should be used to natural bone-like biomaterial, biocompatible, mechanical stable etc. Collagen is one of bone organic matrix occupying, is highly suitable as biomaterials for bone regeneration. However, using alone as scaffold material is not possible because of its poor mechanical property. Therefore, to increase mechanical properties, we fabricated 0, 10, 20, 40, 60, 80 wt% DC/PLGA scaffold by mixing poly(lactic-co-glycolic acid) (PLGA) and collagen extracted from the duck’s flippers (DC) using solvent casting/salt leaching method. The results of evaluating whether or not suitable for bone regenration, it was confirmed that 80 wt% DC/PLGA scaffolds is most excellent bone regeneration capacity in vitro environment. Therefore, we investigated the in vivo environment in this study. In other words, this study evaluated the capability of PLGA and 80 wt% DC/PLGA scaffold when grafted in rat calvarial defect model. Also, Rabbit bone marrow mesenchymal stem cells (rBMSCs) were seeded in scaffolds of two types. At 2, 4, 8 weeks after surgery, 2 and 3 dimensional micro-CT and histological analysis of calvarium was performed in vivo. These results showed that 80wt% DC/PLGA scaffold with rBMSC revealed higher bone regeneration capacity than another group. Thus, 80wt% DC/PLGA scaffold can be envisioned as an useful biomaterials for future bone regeneration applications. This research was supported by Technology Commercialization Support Program (814005-03-2-HD020), Ministry for Food, Agriculture, Forestry and Fisheries, Republic of Korea.

Session No.: P6-207

Evaluation of Osteogenesis from Duck's Feet-derived Collagen/Hydroxyapatite Sponges Immersed Medium with Dexamethasone

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Regenerative medicine has proven to be one of the alternative therapies for diseases and damages of organs. For bone regeneration, some factors are needed such as osteogenic niche, osteogenic differentiation of bone marrow-derived mesenchymal stem cells (BMSCs), high compressive strength of scaffolds, mineralization and so on. Collagen has been widely used as a biopolymers for fabricating scaffolds. Most of collagens are pulled out from tissues of animals because they constitute organs of animals and humans. So, most of collagens have properties of low inflammatory response, high biocompatibility. Dexamethasone is a synthetic glucocorticoid that has been used clinically as an anti-inflammatory drug. It has been reported that BMSCs allowed to proliferate under continuous dexamethasone treatment showed increased osteogenic differentiation. Hydroxyapatite (HAp) is considerably good biomaterial as a bone graft because of its high biodegradable and osteoconductive. In this study, we assessed capacity of osteogenic differentiation of BMSCs on Duck’s feet-derived collagen (DC)/HAp sponge immersed medium with dexamethasone (Dex-DC/HAp). The experimental groups in this study were DC and DC/HAp sponge immersed medium without dexamethasone (DC and DC/HAp group) and with dexamethasone (Dex-DC and Dex-DC/HAp group). We determined the physical and chemical characterizations of DC/HAp sponge through compressive strength, porosity, scanning electron microscopy (SEM), FT-IR and so on. Also, osteogenic differentiation of BMSCs on Dex-DC/HAp sponge was assessed by alkaline phosphatase (ALP) activity assay, reverse transcription-PCR (RT-PCR), MTT assay, and SEM. These results showed that Dex-DC/HAp group increased cell proliferation and osteogenic differentiation of BMSCs during 28 days. From these results, Dex-DC/HAp group will be able to be applied as one of potential biomaterials for bone regeneration. This research was supported by Bio-industry Technology Development Program (112007-05-4-SB010), Technology Commercialization Support Program (814005-03-2-HD020), Rpeublic of Korea.

Session No.: P6-208

Migration Analysis of Osteosarcoma MG-63 Cells on Roughened Substrates Created by Two Photon Polymerization

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Cell migration is important for various osseointegration activities. Many studies based on titanium substrates have confirmed that substrate topography, especially roughness, could affect migration and differentiation of bone cells. Experimental finding suggested that roughened titanium substrates ranging from 1.5 to 4 μm could promote cell migration by two folds. However, due to the roughened substrates can only be created by chemical wet etching process, the uniformity and profile were not easy to control. Further, the sharp ribs and tips were the major interface of the titanium substrate and cells. It could confound the finding of the roughness dependency of cell migration. To understand the role of roughness on the migrating behaviour of bone cells, we use a high precision method, Two Photon Polymerization (TPP), to 3-D lithographically fabricate substrates with controlled and uniform roughness. The profile of roughened surface can be a smooth profile, and we can use it to verify the correlations between roughness and cell migrating velocity. The selected resin for this study was the biocompatible Ormocomp, which provides similar stiffness to bone tissue. Two different roughness were fabricated in this study: 0.4 μm and 1.3 μm. Surfaces of these two substrates were e-beam evaporated with 20 nm titanium as an adhesion layer and another 10 nm SiO2 layer for facilitating fibronectin coating. Osteosarcoma MG-63 Cells were seeded on these substrates, and their migratory behaviours were monitored over 20 hours. The measured migrating velocities of MG-63 cells on substrates with 1.3μm and 0.4 μm roughness were 6.6 μm/hr and 3.48 μm/hr, respectively. Our experimental finding suggested that a roughened substrate without sharp edges could also promote MG-63 cell migration on a SiO2 surface. Our study could potentially identify the role of roughened substrates, and it could be applied to optimize bone healing process.

Session No.: P6-209

Assessment of the Safety of Allogeneic Chondrocyte Sheet Transplantation

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Introduction: We have studied the effects of chondrocyte sheets on the repair and regeneration of articular cartilage. With approval of the Ministry of Health, Labour and Welfare in Japan, we have performed a clinical study to transplant the layered chondrocytes sheets into 8 patients with osteoarthritis. Polydactyly tissue was investigated as a source of candidate cells. Materials and Methods: Safety was assessed using array comparative genomic hybridization (ACGH), G-band straining, and tumorigenesis evaluation in NOG mice. Samples were harvested during surgeries from 12 polydactyly patients (average age 16 months), and chondrocytes were isolated from polydactyly tissue and cultured until passages 2 (P2), P4, P6, and P12. Results: ACGH of genomic DNA in chondrocytes showed no differences in copy number between P2, P4, P6, and P12 cells. G-band staining showed no abnormalities in chondrocytes listed in An International System for Human Cytogenetic Nomenclature(2013). Tumorigenesis evaluation showed no differences in the external appearance and histology at any time point up to 12 weeks of culture. After conducting these investigations and confirming the safety of the polydactyly-derived cartilage cells, we obtained approval from both the university Ethics Committee to conduct clinical research and the Ministry of Health, Labour and Welfare in Japan. Conclusions: We have developed a method to assess the safety of allogeneic chondrocytes sheets, which provide a highly useful method for evaluating allogenic human chondrocytes and other cell types. In future, we plan to use allogeneic chondrocyte sheets to treat more patients.

Session No.: P6-210

Comparative Analysis of Protein Production by Human Chondrocyte Sheets Using a Multiplexed Aptamer-based Assay (Somascan™)

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【Objective】 As we aim for regenerative treatment of knee cartilage using allogeneic chondrocyte sheets, we have examined chondrocytes obtained from polydactyly surgery as an alternative cell source. Secreted protein factors by chondrocyte sheets are likely to be significant contributors to cartilage regeneration; thus in this study, we compared the protein factors produced by chondrocyte sheets to estimate the sheets' in vivo efficacy on cartilage regeneration. 【Methods】 Experiments were performed under the approval and guidance of the Clinical Research Review Committee of Tokai University School of Medicine. The chondrocytes were isolated from cartilaginous tissue from young polydactyly patients (PD: 7 patients; average age = 14.7 months) and patients undergoing surgery for total knee arthroplasty (TKA: 7 patients; average age = 76.1 years). The chondrocyte sheets were created using temperature-responsive culture dishes. The culture supernatants of chondrocyte sheets were analyzed using the SOMAscan™: a multiplexed aptamer-based assay detecting 1129 proteins by slow off-rate modified aptamers (SOMAmers). 【Results】 Statistically significant differences (t-test: P-value < 0.01) between PD sheets and TKA sheets were observed in 211 of 1129 proteins. SOMAscan™ contains 39 of 236 proteins in gene ontology (GO) term GO0051216: cartilage development. Among these 39 proteins, bone morphogenetic protein-10 (BMP-10) and basic fibroblast growth factor (bFGF) were identified as differently produced proteins between PD and TKA sheets. 【Discussion】 Considering that TKA sheets are equivalent to adult chondrocyte sheets that demonstrated their efficacy in our recent clinical study, the difference between TKA sheets and PD sheets is important to consider. The contribution of BMP10 and bFGF to chondrocyte sheets' efficacy on cartilage regeneration should be further studied for application of polydactyly chondrocytes as an alternative cell source.

Session No.: P6-211

Development and Characterization of Oriented Cartilage Extracellular Matrix/Silk Fibroin Scaffold as a Transforming Growth Factor-β3 Delivery System to Promote Chondrogenesis of Adipose-derived Stem Cells

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Self-regeneration capacity of articular cartilage is extremely limited after the damage due to trauma, inflammation or tumor, for which reason cartilage tissue engineering has been a promising approach for cartilage repair. In this study, we developed an oriented cartilage extracellular matrix (CECM)/silk fibroin (SF) composite scaffold. The CECM/SF scaffold was fabricated by using mixed slurry of ultra-homogenized porcine articular CECM and SF with temperature gradient-guided thermal-induced phase separation (TIPS) technique. The scaffold was cross-linked by 1-ethyl-3-3dimethyl aminopropyl carbodiimide (EDAC) and N-hydroxysuccinimide (NHS). After cross-linking, the scaffold was loaded with transforming growth factor-β3 (TGF-β3) by using soaking-lyophilization method and then seeded with rabbit adipose-derived stem cells (ADSCs) to develop cell-scaffold construct. The cell-scaffold construct was cultured in no- TGF-β3-added chemically defined chondrogenic medium (CDM) for 28 days and then evaluated in terms of the physical, biochemical characteristics and the expression of genes related to chondrogenesis. As the comparison task, no-TGF-β3-loaded scaffold was seeded with ADSCs and cultured in CDM to set up positive control group and no TGF-β3 addition group was set as blank control group. It was found that the presence of TGF-β3 could promote superior chondrogenesis of ADSCs and we also observed that the scaffold loaded with TGF-β3 as a delivery system could promote comparable long-term chondrogenesis to the positive control group where the TGF-β3 was continuously added into the medium. The results of this study confirm that oriented CECM/SF scaffold can be used as a TGF-β3 delivery system for cartilage tissue engineering.

Session No.: P6-212

Analysis of Type 1 Diabetes-induced Malformations in Articular Cartilage and Subchondral Bone of Mice

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Although diabetes mellitus (DM) and osteoarthritis (OA) are among the most prevalent disorders, a very little information is available on biology of DM-induced OA. Using steptozotocin (STZ), type 1 diabetic mice model was established and OA-like alterations in knee-joint were assessed. We isolated mice knee-joint from non-diabetic and diabetic origin, named as control and DM-OA group and analysed ultrastructural alterations with hematoxylin and eosin staining while sulfated proteoglycan content was evaluated through safranin 'O' stain. Besides conventional histology, we executed immunohistochemical investigations with type II collagen and carboxymethyl lysine (CML) staining. Bone-matrix associated changes were assessed through alizarin-red S staining. Furthermore, using western blot, the protein expression level of advanced glycation end product (CML), a marker of oxidative stress and inflammatory cytokines (matrix metalloproteinase-1) were evaluated. Chondrogenic markers (SOX9, Col II and AGN) were also analysed. Results showed that CML, and MMP-1 levels were highly increased while SOX9, Col II and AGN were significantly reduced compared to their corresponding controls which are an indicative of osteoarthritis. In conclusion, the diabetes induced catabolic response which led to OA-like symptoms.

Session No.: P6-213

Evaluation of Cartilage Regeneration Using Gellan Gum Based Hydrogel with Hesperdin

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Hydrogels attract great deal of attention in tissue engineering for its resemblance to tissue's extracellular matrix. In addition, the characteristics of which we study Gellan gum hydrogels showed great promise as tunable biomaterials in a wide range of regenerative strategies. These recent studies have led to improvements in Gellan gum properties with the aim of using these new biomaterials for applications in tissue engineering. In this study, Gellan gum based hydrogels were analyzed as cells supports in the context of cartilage regeneration. This study produced different formulations of Gellan gum hydrogels by mixing varying amounts of Hesperidin. The purpose of the present study was to observe the characteristics by seeding chondrocytes with Hesperdin and Gellan gum based hydrogels. The gellan gum based hydrogels with various contents of Hesperidin were characterized by SEM, FT-IR, degradation and water uptake. MTT assay were carried out to confirm cell viability and effect of extra cellular matrix secretion in Hesperidin/Gellan gum hydrogels. This research was supported by grant of the Korea Health Technology R&D Project through the KHIDI (HI15C2996), MOHW, Republic of Korea.

Session No.: P6-214

Evaluation of Low-Acyl Gellan-Gum Hydrogel Containing Chondroitin Sulfate for Articular Cartilage Tissue Engineering: In Vitro Test

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Articular cartilage has an intrinsic difficulty in recovering damages, which requires its tissue engineering treatment. Gellan gum as a natural polysaccharide has good heat resistance, acid resistance and enzymes resistance. Also, Chondroitin sulfate (CS) as the major constituents of cartilage allows rapid diffusion of water-soluble molecules, nutrients and migration of cells. In this study, we fabricate gellan gum hydrogel containing 10%, 20% and 30% chondroitin sulfate (CS) and evaluate possibility of application of cartilage reconstruction. To analyze physicochemical properties and observe morphology of CS/gellan gum hydrogel, compressive strength measurement, DSC, FT-IR and scanning electron microscope (SEM) were carried out. And MTT assay and SEM were performed to measure the proliferation, attachment and viability of cartilage cell in CS/gellan gum hydrogel. Polymerase chain reaction (PCR) was performed to confirm the specific genetic marker of cartilage cells. Though these analyses, we confirmed that 10% CS/gellan gum hydrogel is better than other hydrogels for cartilage regeneration and this hydrogel can be applied to diverse tissue engineering application. This research was supported by grant of the Korea Health Technology R&D Project through the KHIDI (HI15C2996), MOHW, Republic of Korea.

Session No.: P6-215

A Technique to Mimic Synovial Joint Environment for One-step Cartilage Repair

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Articular cartilage has a poor capacity for regeneration and does not restore itself if damaged. Novel strategies include cell-based and tissue engineering techniques to regenerate hyaline cartilage. However, most methods need multiple procedures. A one-step therapy for cartilage repair is attractive because it allows for a minimally invasive procedure and offers a single surgery. In this proof-of-concept study, we assessed the possibility of creating a synovial joint-like microenvironment using articular tissue fragments that would support chondrogenesis. Bone marrow concentrate (BMC), cartilage and synovium fragments were harvested and embedded into fibrin gel to form the biological constructs. Scanning electron microscopic (SEM) images displayed that abundant cells grew and attached on tissue fragments with well-integration in the biological constructs. Histological results also revealed better neo-tissue formations with positive Alcian blue staining. Gene expressions of aggrecan, SOX-9 and type II collagen in the constructs were significantly increased after 6 weeks of in vitro culture. Moreover, the constructs showed good stability and adhesion to defect sites which were confirmed by an ex vivo filling test. In summary, we demonstrated the effect of the biological constructs containing BMC and articular tissue fragments on supporting chondrogenesis. The autogenous BMC and tissues can be easily harvested intra-operatively. This benefits to develop a one-step technique for cartilage repair.

Session No.: P6-216

Regulation of Inflammatory Cytokines in Human Osteoarthritic Chondrocytes by L-Lysine

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Despite the absence of infiltration of neutrophils and macrophages into joint tissues, the levels of inflammatory cytokines and proteases are found to be increased in the synovial fluid of osteoarthritic joint (OA) . Activated fibroblast-like synoviocytes (FLSs) produce interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and matrix metalloproteinases (MMPs). OA synovial fluid activates pro-inflammatory cytokines, alters cell morphology, and causes cell shrinkage in chondrocytes. Chondrocytes also produce cytokines that have autocrine effects, inducing the synthesis of cartilage-degrading proteases and other inflammatory mediators. In response to these cytokines, FLSs further express B cell-activating factor of the TNF family. These factors induce chondrocytes to transform into a hypertrophic phenotype and cause progressive cartilage disruption. Consequently, the severe degradation of articular cartilage induces the apoptotic cell death of chondrocytes. Lysine (Lys) is involved in multiple biological processes including inflammatory regulation. However, rare research has addressed the effects of Lys on human chondrocytes. In this study, chondrocytes were isolated from articular cartilage of OA patients, stimulated withIL-1β and subsequently supplied with Lys. Lys improved hypertrophic transformation of chondrocytes. However, the proliferation of IL-1β-stimulated chondrocytes was still faster than that of unstimulated cells even under providing Lys supplement. The mRNA levels of TNF-α and matrix MMP-9 decreased when normal chondrocytes treated with Lys. IL-1β stimulation upregulated type I collagen (Coll I), Coll X, IL-1β, TNF-α, MMP-3, MMP-9 and downregulated aggrecan, Coll II mRNA levels. On the contrary, Lys down-regulated TNF-α, MMP-3 levels, restored aggrecan and collagens expressions, and further increased the aggrecan/ Coll I and Coll II / Coll I rations in IL-1β-stimulated chondrocytes. In addition, Lys treatment decreased the protein productions of TNF-α and MMP-3 in stimulated cells. Our results suggest that Lys may modulate matrix proteins, inflammatory and catabolic cytokines in OA chondrocytes.

Session No.: P6-217

Glutathione Enhances Antioxidant Capacity of Hyaluronic Acid, Up-regulates Tissue Inhibitor of Metalloproteinase-1 and Interleukin-6 mRNA Expressions in Human Fibroblast-Like Synoviocyte

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Injection of exogenous viscosupplementations into joints reliefs the symptoms of osteoarthritis (OA). Recently, intra-articular therapy with hyaluronic acid (HA) has been widely accepted for the treatment of OA in early stage. Glutathione (GSH), an antioxidant, has been shown to have an anti-inflammatory effect on protecting cells from reactive oxygen species and reactive nitrogen species (ROS/RNS). In this study, the therapeutic effects of HA supplemented with different weight ratios of GSH (0%, 5%, 10% and 20%) on human fibroblast-like synoviocytes (FLSs) were evaluated. The present results showed that cell morphology and glycosaminoglycan production of FLSs were not changed under treatments. However, the addition of 20% GSH decreased cell survival relative to other groups. The total antioxidant capacity was improved and the intracellular ROS/RNS was decreased in treated FLSs. The mRNA expressions of interleukin (IL)-1 beta, tumor necrosis factor-alpha, and matrix metalloproteinase-3 were down-regulated for FLSs cultured in HA or HA+GSH. IL-6 and tissue inhibitor of metalloproteinase-1 (TIMP-1) were up-regulated when HA supplemented with 10% and 20% GSH. In conclusion, the HA supplemented with GSH improves antioxidant capacity, decreases intracellular ROS/RNS, and up-regulates IL-6 and TIMP-1 in FLSs. HA+GSH has the potential to be applied as a new viscosupplementation for OA patients. GSH has the potential to augment the viscosupplementation of HA for OA patients

Session No.: P6-218

Metabolomic Evaluation of the Difference Between Tendon and Ligament in Cellular Level

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Tendon and ligament (T/L) tissues have been known to be obviously different from each other in mechanical properties and ECM production. However, due to the similarities in cellular level such as the overlapping gene markers, distinguishment between T/L fibroblasts has not been clearly verified yet. In this study, we tried NMR-based metabolic profiling of primary T/L fibroblasts to investigate the cellular difference. Primary T/L fibroblasts were harvested from achilles tendon (AT), patella tendon (PT), medial collateral ligament (MCL), and lateral collateral ligament (LCL) by sacrificing New Zealand white rabbits, and then used for RT-PCR to characterize the expression of T/L gene markers; tenomodulin (TNMD), tenascin-C (TNC), collagen type-1 (COL-1) and decorin (DCN). Also, metabolic profiling was performed for each cell group thorough NMR-spectroscopy. All metabolite concentrations were normalized by that of phosphate choline around 3.23 ppm. As a result of RT-PCR, primary T/L fibroblasts remarkably expressed all T/L markers compared to mesenchymal stem cells (MSCs). Tendon groups exhibited distinctly higher level of TNMD expression than ligament groups, but there was no significant difference in expression patterns of TNC, COL-1 and DCN between the two types of cells. In NMR-spectroscopy, primary T/L fibroblasts showed a similar peak pattern on each spectrum. However, several metabolites including lactate around 1.33 ppm, as an important mediator of collagen production in T/L developments represented significantly higher concentrations in tendon cells than in ligament cells. In conclusion, we demonstrated T/L cells are specifically different in metabolite level despite of the ambiguity in gene level. These findings could provide further understanding of the evaluation for T/L regeneration in cellular level. This research was supported by a grant of the Korea Health Technology R&D Project through the KHIDI (HI16C0362, the Ministry of Health & Welfare, ROK) and by the National Research Foundation of Korea Grant (NRF-2014K2A2A7066637).

Session No.: P6-219

Application of Bone Marrow Mesenchymal Stem Cell-derived Extracellular Matrix in Peripheral Nerve Tissue Engineering

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To advance molecular and cellular therapy into the clinic for peripheral nerve injury, modification of neural scaffolds with the extracellular matrix (ECM) of peripheral nerves has been established as a promising alternative to direct inclusion of support cells and/or growth factors within a neural scaffold, while the cell-derived ECM proves to be superior to the tissue-derived ECM in modification of neural scaffolds. In this study, we used bone marrow mesenchymal stem cells (BMSC) as parent cells to generate ECM for application in peripheral nerve tissue engineering. A chitosan nerve guidance conduit (NGC) and silk fibroin filamentous fillers were respectively prepared for co-culture with purified BMSCs respectively, followed by decellularization to stimulate ECM deposition. The ECM modified NGC and lumen fillers were then assembled into a chitosan/silk fibroin-based, BMSC-derived ECM modified neural scaffold, which was implanted into rats to bridge a 10-mm long sciatic nerve gap. Histological and functional assessments after implantation showed that regenerative outcomes achieved by our engineered neural scaffold were better than those by a plain chitosan/silk fibroin scaffold, and suggested the benefits of BMSC-derived ECM for peripheral nerve repair.

Session No.: P6-220

Human Pluripotent Stem Cell (PSC)-derived Mesenchymal Stem Cells (MSCs) Show Potent Neurogenic Capacity Which Is Enhanced with Cytoskeletal Rearrangement

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Mesenchymal stem cells (MSCs) are paraxial mesodermal progenitors with potent immunomodulatory properties. Reports also indicate that MSCs can undergo neural-like differentiation, offering hope for use in neurodegenerative diseases. However, ex vivo expansion of these rare somatic stem cells for clinical use leads to cellular senescence. A newer source of MSCs derived from human pluripotent stem cells (PSC) can offer the 'best-of-both-worlds' scenario, abrogating the concern of teratoma formation while preserving PSC proliferative capacity. PSC-derived MSCs (PSC-MSCs) also represent MSCs at the earliest developmental stage, and we found that these MSCs harbor stronger neuro-differentiation capacity than post-natal MSCs. PSC-MSCs express higher levels of neural stem cell (NSC)-related genes and transcription factors than adult bone marrow MSCs at baseline, and rapidly differentiate into neural-like cells when cultured in either standard neurogenic differentiation medium (NDM) or when the cytoskeletal modulator RhoA kinase (ROCK) is inhibited. Interestingly, when NDM is combined with ROCK inhibition, PSC-MSCs undergo further commitment, acquiring characteristics of post-mitotic neurons including nuclear condensation, extensive dendritic growth, and neuron-restricted marker expression including NeuN, β-III-tubulin and Doublecortin. Our data demonstrates that PSC-MSCs have potent capacity to undergo neural differentiation and also implicate the important role of the cytoskeleton in neural lineage commitment.

Session No.: P6-221

NGF and Extremely Low-frequency Electromagnetic Fields Induce Neuronal Differentiation of PC12 Cells

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Recently, extreme low-frequency magnetic fields (ELF-MFs) can influence on various cellular events, such as gene expression, cell cycle, cell proliferation, differentiation, and even cell fate. Many studies have been reported that PC12 cells, a pheochromocytoma cell line from rat adrenal medulla, are sensitive to neuronal growth factor (NGF) and obviously they can differentiate to neuronal cells with NGF. This study aimed to confirm that the effect of ELF-MFs can induce neuronal differentiation of NGF induced PC12 cells. We observed that the levels of mRNA and protein which are related neuronal differentiation improved in NGF and ELF-MFs induced PC12 cells. We examined PC12 cells with NGF and ELF-MFs expressed different protein pattern from that of PC12 cells with NGF only using two-dimensional electrophoresis (2-DE) gels. After detecting these 6 different spot between the 2-DE maps, we performed electrospray ionization quadrupole time-of-flight tandem mass spectrometry (ESI-QTOF LC/MS/MS) to identify 6 kinds of proteins.We are anticipating that this identification of proteins can provide how ELF-MFs work on PC12 cells differentiated with NGF and furthermore how to find a brand new way to treat neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease and multiple sclerosis.

Session No.: P6-222

Joint Use of Chitosan–PGA Nerve Guidance Conduits and Bone Marrow Mesenchymal Stem Cells (MSCs) to Repair 50mm Long Median Nerve Defect Combined 80mm Long Ulnar Nerve Defect in the Human Upper Arm

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Purpose: This case study was performed to observe the repair outcomes and safety after surgical repair of long-distance peripheral nerve defect in the upper arm by implantation of chitosan–PGA nerve guidance conduits combined MSCs. Methods: A 29 year-old woman, who was injured with 50mm long median nerve defect and 80mm long ulnar nerve defects combined the muscle and blood vessel disruption in the right upper arm. The nerve defect were repaired by implantation of chitosan-PGA nerve guidance conduits joint MSCs in the 40th day after injury. After surgical repair, a series of functional assessments were carried out in different times. Motor function was evaluated by the recovery of the joint movement and strength which supplied by the injured nerves. Sensory recovery was assessed by two-point discrimination and touch test with Semmes-Weinstein monofilament. Autonomic function was monitored by Laser Doppler perfusion imaging (LDPI). We observed changes of nerve conduits and nerve regeneration in human body through MRI, ultrasonic and electrophysiological. Blood and urinary routine tests, serum biochemical examinations were taken to monitor the safety of the nerve graft after surgery. Results: 1 year post-implantation, the cutaneous blood perfusion of the right hand increased. the range of the wrist flexion increased. pain test and touch test with monofilament of the finger restored. the results indicated that the function of the injured median nerve is in recovery, but the function supplied by ulnar nerve didn't improve. MRI and ultrasonic showed nerve conduits were absorbed and the atypical regeneration of nerve fibers. No anomalies in blood and urinary routine and serum biochemical examinations were detected. No complications were observed. Conclusions: The results indicate that as an alternative to nerve autografts, chitosan–PGA nerve guidance conduits combined MSCs could be a effective and safe choice for repairing extended nerve defects.

Session No.: P6-223

Evaluation of Peripheral Nerve Regeneration in Diabetic Rats Using Current-modulated Electrical Stimulation

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To study if electrical stimulation (ES) can be a useful tool to improve functional recovery after neuronal injury in the peripheral nervous system. We studied the effects of 2 Hz of percutaneous ES at different intensities of 1, 10, and 20 mA on peripheral nerve regeneration in rats with diabetes induced by streptozotocin. Non-stimulated diabetic rats were used as the sham-controls. The animals were divided into five groups. Group A, nondiabetic normal animals underwent the nerve lesion and repaired with empty silicone rubber chambers. In group B, diabetic animals were the sham-controls, which received empty silicone rubber chambers and the stimulator did not deliver current to the two stainless steel needle electrodes. Groups C-E, diabetic animals received a treatment of electrical stimulation of 2 Hz at current levels of 1, 10, and 20 mA, respectively, after their injured nerves were bridged with the silicone rubber tubes. A10-mm gap was made in the rat sciatic nerve by suturing the stumps into silicone rubber tubes and stimulation was carried out every other day for 3 weeks starting 1 week after surgery. After 4 weeks of recovery, the diabetic rats showed that ES of 1 mA or above could increase the cutaneous blood flow in their ipsilateral hindpaw to the injury. ES of 10 mA could improve the amplitude and the area of evoked muscle action potentials with faster target muscle reinnervation. ES of 10 mA could also ameliorate the calcitonin gene-related peptide expression in lamina I-II regions in the dorsal horn ipsilateral to the injury and the number of macrophages in the diabetic distal sciatic nerve. The impaired growth and maturation of regenerating axons in diabetic rat could be improved by ES of 10 mA or above.

Session No.: P6-224

Porous Micropatterned Nerve Guidance Conduits Enhanced Neural Stem Cell Alignment and Sciatic Nerve Regeneration

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Tissue engineered nerve guidance conduits (NGCs) has been designed highly aligned architecture as mimetically native tissues essential for efficient regeneration of nerve injuries. For successful nerve regeneration, we developed a biodegradable poly(lactic-co-glycolic acid) (PLGA) NGCs with longitudinally oriented micropatterned inner surface by combining capillary force lithography and rolling the substrate methods. In addition, we converted interconnected porous NGCs by using salt leaching methods that requires nutrients and oxygen infiltration for nerve tissue regeneration and survival of migrated host neural cells. In this study, we observed that the orientation of neurite in mouse neural stem cells was regulated in micropatterned surfaces compare to the flat substrates. Also, we evaluated that porous micropatterned substrates have promote longitudinal orientation and neurite extension in mouse neural stem cell. Thus, we fabricated porous patterned NGCs with immobilization of 3,4-dihydroxy-L-phenylalanine (DOPA) and implanted in 10 mm gap of rat sciatic nerve. In 4 weeks, the number of neurofilament per millimeter square in central region of both NGC and DOPA-NGC group showed similar density compare to normal nerve tissue. Additionally, DOPA-NGCs group showed higher sciatic function index and CMAP amplitude compare to NGC group. Based on these results, we suggest that this porous micropatterned NGCs could potentially be useful for injured nerve tissue guidance and regeneration in the peripheral nerve or spinal cord injuries.

Session No.: P6-225

Wortmannin Has the Reno-protective Effect on Streptozotocin-induced Proteinuric Renal Disease Rats in Vivo

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This study aimed to investigate the reno-protective effect of wortmannin, the phosphoinositide 3-kinase (PI3K) inhibitor, to treat early diabetic nephropathy (DN). DN is a kidney disease caused by damage to the kidney glomeruli. Podocytes, glomerular epithelial cells, have numerous functions in the glomerular filtration barrier. It has been shown that it is important to regulate the podocyte actin cytoskeleton in early DN. Wortmannin simultaneously regulates GTPases such as Rac1 and Cdc42; therefore those proteins destabilize the podocyte actin cytoskeleton during early DN. We treated wortmannin on streptozotocin-induced proteinuric renal disease (SPRD) rats to inspect the reno-protective effects of wortmannin by lowering albuminuria and albumin to creatinine ratio. In addition, histopathological changes in kidney sections of the SPRD group also indicate that wortmannin has reno-protective effects. We confirmed the expression levels of proteins related to actin cytoskeleton in podocytes such as nephrin, podocin, and Rac1/Cdc42 in the wortmannin group were greater than those in the SPRD group by Western blotting. Furthermore, the localization and expression of nephrin, podocin, and desmin in the kidney glomeruli were revealed by immunofluorescence. Those results suggest that wortmannin has a reno-protective effect on SPRD rats and this compound is a candidate as an agent for delaying the initial stage of DN.

Session No.: P6-226

Bacterial Analysis of Periodontal Guided Tissue Regeneration Infection in Dogs

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Bacterial analysis of periodontal guided tissue regeneration infection in dogs YANG Qiu-Bo , SUN Xiao-Ni ,LIU Rui-Jie, LIU Ying and Zhang Zi-Lu Beijing Institute for Dental Research， Capital Medical University School of Stomatology，Beijing 100050，China Corresponding author: YANG Qiu-Bo，Email: qiuboyang2003@163.com Objective The purpose of this study was to investigate characteristic of the bacteria population involved in infection of periodontal guided tissue regeneration (GTR)· Methods Class II furcation defects were created in the first and second premolars of 2 beagle dogs. Space-providing expanded polytetrafluoroethylene (ePTFE) membranes were implanted to provide for GTR · The ePTFE membranes were retrieved 8 weeks after GTR for detecting the species and population of bacteria on ePTFE membranes and alveolar bone by selective and non-selective culture · Results Fusobacterium nucleatum, Actinobacillus actinomycetemcomitans, Black-pigmented bacterium, Actinomycete, and Streptococcus mutans were detected from both of ePTFE membranes and alveolar bone · Alveolar bone showed higher population of black-pigmented bacterium compared with ePTFE membranes. Conclusion Our findings implied that a variety of bacteria could be involved in infection in periodontal guided tissue regeneration. Antibacterial agent that has abroad-spectrum of antimicrobial activity should be selected to prevent periodontal GTR infection. Key words: Guided tissue regeneration; Polytetrafluoroethylene membrane, Infection This work was supported by grants from the National NaturalScience Foundation of China (Grant 30572037, 81470753) and the Beijing Natural Science Foundation (Grant 7062028, 7122078).

Session No.: P6-227

Extracellular Matrix and Effect of Ca2+ Signaling on the Muscle Remodeling

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The main aim of the presentation is to reconstruct the skeletal muscle organ using ECM scaffold. Since acellular biological matrix can be used only as a passive element (e.g. bridge) that is colonized from outside by adjacent recipient cells (that lack any navigation to enter the graft) we plan to colonize the graft with donor-derived cells immediately prior graft implantation. We hypothesize the combination of myogenic and non-myogenic cells can be optimal for stimulating ingrowth of recipient cells responsible for graft vascularization, innervation and infiltration with inflammatory cells (that initiate the tissue repair). The resulting interaction between recellularized scaffold and recipient cells will support remodelling of the scaffolds microenvironment through partial disruption of basal laminas, local change in ECM composition and reduced stiffness which are critical prerequisites for successful muscle regeneration. We will evaluate the general hypothesis that cellular Ca2+ signals control the physiopathological properties of myogenic and non-myogenic cells before and after implantation. Further, we predict that the specificity of cellular action depends on the source, localisation and mechanism of Ca2+ signal generation and can be used as a predictive factor for selection of optimal cells for scaffold recellularization. This work was supported by grant GACR No 15-09161SR and the programme PRVOUK 37/06.

Session No.: P6-228

The Tissue-protective Effect of PRP on the Recovery of Erectile Function Mediated Partly by Action of Cytokine CXCL5

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Platelet rich plasma (PRP) contains high concentrations of growth factors, and demonstrated its effects on erectile function recovery after bilateral cavernous nerve (CN) injury in a rat model. The purpose of the present study was to identify the potential neurotrophic and cell-protective factors in PRP that promote the recovery of neurogenic erectile dysfunction. We determined the concentration of neurotrophic factors in PRP and plasma by cytokine array and enzyme-linked immunosorbent assay (ELISA). The cytokine antibody array ELISA results showed that the PRP contained higher levels of CXCL1, CXCL5 and CCL2. We determined the expression patterns of CXCL1 and CXCL5 and their receptors in the major pelvic ganglion (MPG) and corpus cavernosum via immunostaining. We then evaluated the protein expression of these cytokines and their receptors in the MPG and CC 3 d, 7 d, and 14 d post-CN injury and PRP treatment, and in the controls. In addition, these genes also were evaluated at 2 h, 8 h, and 1 d post-injury and PRP treatment. We found that CXCL1 and CXCL5 were expressed strongly and CXCR2 was expressed weakly in the surface of the neurons of the MPG. The intracavernous injection of PRP may promote the stable expression of CXCR2 and increase the expression of CXCL5 and CXCL1 in the MPG post CN injury, and enhance the ability of neurogenic recovery. We also found that the intracavernous injection of CXCL5 promoted recovery from CN injury-induced erectile dysfunction by preventing smooth muscle atrophy. In conclusion, PRP's therapeutic efficacy post CN injury may be a synergistically action with other multiple biomolecules and partly mediated by action of cytokine CXCL5. The endpoint of our study attempted to support the optimization of the PRP formulation to provide safe and effective medications to patients with radical prostatectomy for the recovery of erectile function.

Session No.: P6-229

Enhanced Bone Tissue Regeneration by Porous Gelatin Composites Loaded with the Chinese Herbal Decoction Danggui Buxue Tang

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Danggui Buxue Tang (DBT) is a traditional Chinese herbal decoction containing Radix Astragali and Radix Angelicae sinensis. Pharmacological results indicate that DBT can stimulate bone cell proliferation and differentiation. The aim of the study was to investigate the efficacy of adding DBT to bone substitutes on bone regeneration following bone injury. DBT was incorporated into porous composites (GGT) made from genipin-crosslinked gelatin and β-triclacium phosphates as bone substitutes (GGTDBT). The biological response of mouse calvarial bone to these composites was evaluated by in vivo imaging systems (IVIS), micro-computed tomography (micro-CT), and histology analysis. IVIS images revealed a stronger fluorescent signal in GGTDBT-treated defect than in GGT-treated defect at 8 weeks after implantation. Micro-CT analysis demonstrated that the level of repair from week 4 to 8 increased from 42.1% to 71.2% at the sites treated with GGTDBT, while that increased from 33.2% to 54.1% at GGT-treated sites. These findings suggest that the GGTDBT stimulates the innate regenerative capacity of bone, supporting their use in bone tissue regeneration.

Session No.: P6-230

Maintained Stemness of Human Periodontal Ligament Stem Cells Following Prolonged Storage of Extracted Teeth

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Human periodontal ligament stem cells (hPDLSCs) are readily accessible and promising mesenchymal cell source for therapeutic strategies. For clinical applications of stem cell therapies, prolonged maintenance of the hPDLSC stemness is critical. The optimization of cell quality and standardization of manufacturing protocols require an evaluation of hPDLSC characteristics after storage. The purpose of the present study is to evaluate the maintenance of hPDLSC stemness following storage of the periodontal ligament (PDL) in growth, proliferation, and differentiation capabilities. Following premolar extraction (N=10), hPDLSCs were isolated immediately (n=5) and after 1 week (n=5) of tooth storage in a growth medium, followed by hPDLSC primary culture. The hPDLSCs were evaluated in colony-forming and proliferative abilities, immunophenotypes and differentiation capabilities (osteogenic, adipogenic, and chondrogenic) followed by real-time polymerase chain reaction (PCR) confirmation. The results showed that storing the PDL did not affect the stemness of hPDLSCs. The hPDLSC activities from the PDL immediately after harvest and following 1 week of PDL storage were comparable in colony-formation and proliferation capabilities, and immunophenotypes. The osteogenic, adipogenic, and chondrogenic differentiation capabilities of the hPDLSCs were unaltered after PDL storage. The real-time PCR analyses confirmed the maintenance of relative gene expression levels for osteogenic (RUNX2, ALP, and OCN), adipogenic (PPAR-γ and LPL), and chondrogenic (aggrecan and collagen types 2 and 10) differentiations. Within the limitation of the study, the hPDLSCs harvested immediately from the PDL and following 1-week PDL storage were shown to have maintained the important characteristics of mesenchymal stem cells. *Acknowledgement: This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (NRF-2015R1A2A2A01004589).

Session No.: P6-231

Functional Modification of Fibrous PCL Scaffolds with Fusion Protein VEGF-HGFI Enhanced Cellularization and Vascularization

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The lack of efficient vascularization within frequently-used Poly(ε-caprolactone) (PCL) scaffolds has hindered their application in tissue engineering. VEGF is known as one of the most prominent pro-angiogenic growth factors and has been widely utilized to induce vascularization. Hydrophobin HGFI, an amphiphilic protein, can form a self-assembly layer on the surface of PCL scaffolds and convert their wettability. In this study, a fusion protein consisting of hydrophobin HGFI and VEGF was prepared by Pichia pastoris (P. pastoris) expression system. SDS-PAGE and Western blotting confirmed that the fusion protein VEGF-HGFI was successfully isolated and purified. Transmission electron microscope (TEM) and water contact angle (WCA) measurement demonstrated that VEGF-HGFI could form a self-assembly layer with about 25 nm in thickness on electrospun PCL fibers and increase their hydrophilicity. Scanning electron microscope (SEM) and mechanical measurement indicated that VEGF-HGFI modification had no impact on morphology and mechanical property of electrospun PCL scaffolds. VEGF-HGFI functional modification could effectively enhance the adhesion, migration and proliferation of human umbilical vein endothelial cells (HUVECs). NIR fluorescence imaging showed the Cy7-labeled VEGF-HGFI functional modification on PCL scaffolds could exist in PBS under shaking for at least 21 days and in subcutaneous implantation for at least 14 days. Bioluminescence imaging (BLI) showed that VEGF-HGFI could effectively activate VEGFR2 receptors. Subcutaneous implantation in mice and rats revealed that cellularization and vascularization were significantly improved in VEGF-HGFI modified PCL scaffolds. These results suggest that the fusion protein VEGF-HGFI is a useful molecule for functional modification of scaffolds to enhance cellularization and vascularization in tissue engineering.

Session No.: P6-232

Novel Skin Chamber for Rat Ischemic Flap Studies in Regenerative Wound Repair

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Background: In plastic surgery, skin flap is an important approach to reconstructive wound repairs. The rat dorsal skin flap is a clinically relevant and popular animal model to investigate and evaluate flap survival and necrosis. Nonetheless, flap survival is often unstable with unpredictable outcomes, regardless of previous attempts at design modification. Methods & Results: In the current study, we report a novel flap chamber that provides stable and reproducible outcomes by separating the dorsal skin flap from its surrounding skin by in situ immobilization. The flap chamber blocks circulation that disturbs flap ischemia from both basal and lateral sides of the flap tissue. Demarcation of skin necrosis is macroscopically evident on the flap and supported by distinct changes in histological architecture under microscopic examination. The utility of the novel skin flap chamber is further proven by applying it to the examination of flap survival in streptozotocin-induced diabetic rats with an increase in skin necrosis. The flap chamber also affords size modifications where a narrower flap chamber increases ischemia and provides manipulable therapeutic windows for studying cell therapies. Accordingly, intradermal injection of endothelial cells 3 days before flap ischemia significantly increases the survival of skin flaps. Conclusions: The novel flap chamber may not only stabilize the skin flap and provide reproducible outcomes that overcome the shortfalls of the traditional ischemic flap, but may also afford size modifications that support research designs and test therapeutic approaches to regenerative repair.

Session No.: P6-233

Coacervate-coated Nanofiber Based Delivery of Dual Growth Factors for Improvement of Skin Defect

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Random skin flap surgery procedures are frequently used in plastic and reconstructive surgery to repair the local defects. However, distal end necrosis impedes the viability of skin flap due to poor perfusion of blood. Delivery of angiogenesis growth factors (GFs) improved the survival of skin flap through neovascularization. In this study, dual growth factors (VEGF and TGF-β3) were delivered through a coacervate-coated nanofibrous scaffold. The preparation of coacervate is a simple process by mixing of polycation (i.e., poly(ethylene argininylaspartate diglyceride)) (50 µl), heparin (10 µl), and cargo growth factors (2 µl). The electrospun PLGA nanofibers were punched and sterilized through 70% EtOH for 1 hr under UV in hood then nanofibers were washed several times with PBS. Coacervates were coated on electrospun-PLGA and further dried for 30 minutes at 37 ℃. Coacervate coating on nanofibers was confirmed through SEM and ATR-FTIR. VEGF and TGF-β3 loaded coacervate coated nanofiber showed a relatively sustained release of VEGF and a rapid release of TGF-β3, especially for the first 3 days. The tubule formation assay showed an extended network of tubules in dual growth factors encapsulated in coacervate as compared to all other groups. By using a rat skin flap model, it was demonstrated that dual GF delivery using coacervate-coated nanofibers indicated a significantly higher area of flap survival as compared to the control. Similarly, the Doppler and immunohistochemistry (IHC) data also showed the significantly facilitated blood perfusion and enhanced the number of vessels in dual GF group. Taken together, our platform for GF delivery has a therapeutic potential for wound healing and survival of skin flap.

Session No.: P6-234

Epidermal Growth Factor (EGF)-Like Repeats and Discoidin I-Like Domains 3 (EDIL3): A Potential New Therapeutic Tool for the Treatment of Keloid Scars

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In keloids, the mechanism underlying the excessive accumulation of extracellular matrix after injury of the skin is unclear, and there is no effective treatment because of the incomplete understanding of their pathogenesis; thus, a high recurrence rate is observed. We studied a new marker of keloids to determine a new treatment strategy. First, the keloid gene expression profile (GSE44270) was analyzed (downloaded from the Gene Expression Omnibus database) and the new keloid marker candidate, epidermal growth factor (EGF)-like repeats and discoidin I-like domains 3 (EDIL3) which were upregulated in keloid samples was identified. EDIL3 is a unique ligand of $\alpha v \beta 3$ integrin, which is generated by endothelial cells, and this integrin is a part of a secretion signaling pathway of the cell. By binding to $\alpha v \beta 3$ integrin, EDIL3 can modulate apoptosis to facilitate the attachment of endothelial cells. Knockdown of EDIL3 is known to suppresses angiogenesis by downregulating relevant inhibitory factors that can limit the supply of survival factors to tumor cells from the circulation via the vascular endothelial cells. In keloids, the mechanism of action of EDIL3 may be similar to that in tumors; the inhibition of apoptosis in tumor cells via a reduction in the apoptosis of blood vessels by upregulating an angiogenic factor. To determine whether EDIL3 is involved in keloid formation, we performed knockdown of EDIL3 in keloid fibroblasts in vitro by transfection with anti-EDIL3 small interfering RNA (via microporation). EDIL3 was upregulated in keloid fibroblasts compared with normal fibroblasts in collagen type I, II and Ⅲ. Our results indicate the control of EDIL3 expression may be a new promising treatment of keloid disease also the molecular targeting of EDIL3 may improve the quality of treatment and reduce the formation of keloids.

Session No.: P6-235

Small-diameter Vascular Grafts for Simultaneous Regeneration of Smooth Muscle and Endothelium

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Small-diameter vascular grafts are of great clinical needs because of increasing cardiovascular disease (CVD). In vivo tissue engineering, as an effective approach to regenerate small-diameter blood vessel, is attracting considerable attention. However, simultaneous regeneration of smooth muscle and endothelium remains a great challenge. To this end, we fabricate bi-layered small-diameter vascular grafts by combining wet-spinning and electrospinning techniques. In this bilayers vascular graft, wet-spun poly(glycolide-co-lactide-co-caprolactone)(PGLC) with circumferentially aligned microfibers is used as the inner layer and electrospun poly(ε-caprolactone)(PCL) with random nanofibers as the outer layer. We investigated firstly the difference of the in vivo degradation rate between PGLC and PCL through subcutaneous implantation in rats and analyzed by GPC and loss weight. Then we evaluated the ability of vascular graft to regenerate smooth muscle and endothelium in vivo by implanting the tubular graft in the rat abdominal aorta for 2, 4 and 12 weeks. We found the PGLC microfibers began to break after two weeks of subcutaneous implantation and there is no full fibers exists after three months. The average molecular weight decreased with implantation time, and mass loss occurred. The results of implantations in vivo revealed that the vascular graft maintained well-shape. There was no thrombosis after 2, 4 and 12 weeks implantation. Patency rate reached 100% in 25 rats. Immunological analysis demonstrated that a confluent endothelium layer was formed and covered the entire inner surface of vascular graft. A large number of smooth muscle cells were found to exist inside of tubular graft and adopt a circumferential fashion at three time points. Histological analysis showed that extracellular matrix (ECM) related to smooth muscle including collagen (I, II) and elastin were expressed. In conclusion, we provided a new and very useful strategy to fabricate small-diameter vascular graft which can achieve simultaneous regeneration of smooth muscle and endothelium.

Session No.: P6-236

Cell-penetrable Microfibrous Matrices Using Simple Fluidic Device and Precipitation Method for Tissue Engineering

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Microfibrous porous matrices were fabricated from poly(ε-caprolactone) (PCL) using a simple fluidic device and precipitation method. Organic PCL solution and ethanol were introduced into the simple fluidic device as the discontinuous and continuous phases, respectively. PCL polymer was precipitated at the tip of the needle, leading to a microfibrous structure in the collection phase (ethanol). The high flow rate of the continuous phase led to the reduction in the microfiber diameter. Confocal microscopy images revealed that the highly porous structure enough for cell penetration. Cell culture assay confirmed a faster rate of cell proliferation on the microfibrous matrix compared with the electrospun-fibrous matrix. The microfibrous matrix has a great potential for tissue engineering.

Session No.: P6-237

The Efficacy of Tissue Engineering Skin in Applying the Healing of Chroinic Ulcers

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The Efficacy of Tissue Engineering Skin in Applying The Healing of Chroinic Ulcers Kun Song1,2, Jie Luo1,2, Lu Yang1, 2, Yongjie Zhang1 Extensive defect of skin is one of common and intractable diseases in clinic, people desired to find ways to resolve the problem from early ago. Patients with chronic skin ulcer caused skin defect, surrounding tissue and cell dysfunction, insensitive to conventional therapies, due to systemic or local factors such as excessive blood sugar, ischemia of extremity end, nervous lesion, abnormal immunity and infection. During clinical observation patients with of wound infection, gangrene, long course, even amputation will suffer unacceptable pain. This good clinic application method of two layers tissue engineered active skin (ActivSkin) on the great scale diabetic ulcer are discussed in the paper. The two layers tissue engineering active skins were prepared using the epidermal cells and fibroblasts from the same race as epidermal seeds cells and dermal seeds cells · The chronic ulcer wound could be repaired by tissue engineering skin in prompting the early healing, shortening the ill course and reducing its complications · It is practical and feasible for clinical application of tissue engineering skin. Key words: Tissue engineering skin; Chronic skin ulcer; Clinical treat 1, Xi'an Institute of tissue engineering and regenerative medicine, Xi’ an 710032 2, Shaanxi Bio-Regenerative Medicine Co., Ltd., Xi’ an 710025

Session No.: P6-238

The Use of Tumor Cell-conditioned Medium for Therapeutic Angiogenesis

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The use of tumor cell-conditioned medium for therapeutic angiogenesis Hyeon-Ki Jang1, Jin Han2, Jeong-Kee Yoon2, Ju-Ro Lee2, Gun-Jae Jeong2, Jung-Youn Shin2, Byung-Soo Kim1,2 1Interdisciplinary Program for Bioengineering, Seoul National University, Seoul 151-744, Republic of Korea 2School of Chemical and Biological Engineering, Seoul National University, Seoul 151-744, Republic of Korea Stem cell-conditioned medium (CM), which contains angiogenic factors that are secreted by stem cells, represents a potential therapy for ischemic diseases. Along with stem cells, tumor cells also secrete various angiogenic factors. Here, tumor cells as a cell source of CM for therapeutic angiogenesis was evaluated and the therapeutic efficacy of tumor cell CM in mouse hindlimb ischemia models was demonstrated. CM obtained from a human fibrosarcoma HT1080 cell line culture was compared with CM obtained from a human bone marrow-derived mesenchymal stem cell (MSC) culture. HT1080 CM contained higher concentrations of angiogenic factors compared with MSC CM, which was attributable to the higher cell density that resulted from a much faster growth rate of HT1080 cells compared with MSCs. For use in in vitro and in vivo angiogenesis studies, HT1080 CM was diluted such that HT1080 CM and MSC CM would have the same cell number basis. The two types of CMs induced the same extent of human umbilical vein endothelial cell (HUVEC) proliferation in vitro. The injection of HT1080 CM into mouse ischemic limbs significantly improved capillary density and blood perfusion compared with the injection of fresh medium. Although the therapeutic outcome of HT1080 CM was similar to that of MSC CM, the preparation of CM by tumor cell line culture would be much more efficient due to the faster growth and unlimited life-time of the tumor cell line. These data suggest the potential application of tumor cell CM as a therapeutic modality for angiogenesis and ischemic diseases.

Session No.: P6-239

The Development of a Novel Bioabsorbable Implant That Is Substituted by Adipose Tissue in Vivo

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Adipose tissue has recently been regenerated by combining bioabsorbable scaffolds, growth factors and/or adipose tissue-derived stromal cells (ASCs). However, the safety of growth factors and ASCs have not been confirmed in cancer patients. We reported that adipose tissue was regenerated in the internal space of a polypropylene mesh impregnated with collagen sponge (CS) without growth factors or cells. In this study, we explored the formation of adipose tissue using bioabsorbable implants containing CS in rodent models. We prepared implants with/without CS using poly L-lactide-co-ε-caprolactone (P[LA/CL]) or poly-L-lactic acid (PLLA) threads. We confirmed the compressive strength of these implants. In the animal experiments, we set the following seven groups and performed the procedure in the inguinal region of the rat. In the control group, no operative procedure was performed. In the sham operation group, skin incision and undermining without implantation was performed. The CS or four other types of implant were implanted in the CS group, P(LA/CL) group, P(LA/CL) with CS group, PLLA group and PLLA with CS group. The areas of formed tissue and adipose tissue inside the implants were evaluated. All implants had sufficient strength to maintain the internal space before implantation. The internal space was maintained for up to 12 months and adipose tissue was formed in two PLLA groups. At 6 months, the internal space was maintained and more adipose tissue was formed in the PLLA with CS group than in the PLLA group. The adipose tissue of the PLLA with CS group increased from 3 to 6 months. We showed that the PLLA implant is a novel bioabsorbable implant that maintains the internal space for one year and regenerates adipose tissue without growth factors or cells. PLLA with CS seems more effective with regard to the formation of adipose tissue in the short-term than PLLA alone.

Session No.: P6-240

Repair of Large Size Osteochondral Defects in Articular Weight-bearing Areas with Autologous BMSC Engineered Cartilage in Vitro in a Swine Model

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Functional reconstruction of large osteochondral defect is always a major challenge in articular surgery. A few studies have reported the feasibility of repairing articular osteochondral defects based on bone marrow stromal cells (BMSCs) and biodegradable scaffolds. However, no significant breakthroughs have been achieved in clinical translation due to the disadvantages of direct implantation with cell-scaffold constructs such as cell leakage, insufficient mechanical property, inflammatory reaction caused by scaffolds, and unstable cartilage regeneration in vivo. The current study proposed that in vitro cartilage regeneration strategy, which provided relatively mature cartilage-like tissue with superior mechanical property, may help to overcome the above disadvantages and achieve satisfactory repair. The current strategy involved in vitro cartilage engineering, evaluations of engineered cartilage, repair of osteochondral defects with autologous BMSC engineered cartilage, and evaluations of in vivo repair efficacy. The results demonstrated that BMSC engineered cartilage in vitro (BEC-vitro) presented a time-depended mature course with degradation of scaffolds. Furthermore, BEC-vitro at 4 and 8 weeks successfully repaired large size osteochondral defects at articular weight-bearing areas and achieved much better outcome compared to BMSC-scaffold constructs (2-week group). Most importantly, the implantation of BEC-vitro alone realized tissue-specific repair of osteochondral defects with both cartilage and subchondral bone along with ideal interface integration. These results indicated that in vitro cartilage regeneration of BMSCs was a promising strategy for functional reconstruction of large size osteochondral defect and thus helped to promote the clinical translation of cartilage regeneration techniques based on BMSCs.

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